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(57) Abstract

The present invention relates to methods for identifying nucleic acid sequences which encode two or more specific interacting peptides or proteins. Furthermore, the present invention relates to kits which may be used for the identification of nucleic acid sequences in accordance with the method of the present invention.

General description of the polyphage principle
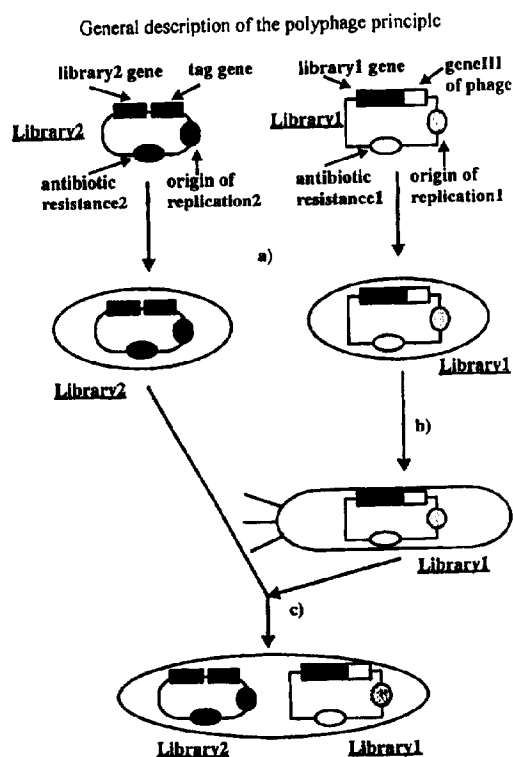
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NOVEL METHOD FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING TWO OR MORE INTERACTING (POLY)PEPTIDES

The present invention relates to methods for identifying nucleic acid sequences which encode two or more specific interacting peptides or proteins. Furthermore, the present invention relates to kits which may be used for the identification of nucleic acid sequences in accordance with the method of the present invention.

Protein-protein interactions play an important role in all biological processes, from the replication and expression of genes to the morphogenesis of organisms (Lewin, B. 1994, Genes V. Oxford University Press). Methods for detecting protein-protein interactions have proved useful in understanding the basic mechanisms of different biological processes and the development of therapeutics. Detection of protein-protein interactions can be divided into two main categories: (i) physico-chemical based and (ii) genetic approaches (Phizicky, E.,M. & Fields, S. Microbiological Reviews 59 (1995) 94-123). Detection of protein-protein interactions by physico-chemical methods usually requires significant amounts of material, and more importantly, the identity of the proteins to be studied must be known. Recent developments in methods of mass spectrometry circumvent this problem but such suffer the disadvantage of requiring sophisticated equipment and expertise (Wang, R. & Chait, B.T., Current Opinion in Biotech. 5 (1994) 77-84). In contrast, genetic approaches provide an easy and powerful method of identifying protein-protein interactions without the need for pure material and specialized equipment, with the added advantage of higher throughput.

Different genetic approaches have been used to identify protein-protein interactions. The current method of choice is the yeast 2-hybrid system (Fields, S. & Song, O.K.,

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Nature (London) 340, (1989) 245-246) which allows the identification of novel proteins that interact with a known protein.

Another popular genetic approach is the phage display system (Patent Application WO90/02809) whereby proteins are fused to a component of a surface protein of filamentous phage to allow selection for binding to a ligand of interest. The gene encoding the protein displayed on the surface of the phage is packaged inside the phage allowing the coupling of genetic information with the gene product. This allows the screening of "libraries" of proteins whereby the identity of the screened protein is deduced from the nucleic acid sequence of the phage. This technique has been extended by Winter et al. (Patent Application WO 92/20791) to produce libraries of multimeric members of a specific binding pair (e.g. combinations of VH and VL chains of an antibody) and select for functional specific binding pair members that can bind to the complementary specific binding pair member (e.g. antigen). Said libraries are constructed by combining two sub-libraries each encoding a collection of corresponding sub-units of said multimeric members (e.g. a library of VH chains is combined with a library of VL chains) wherein in principle each sub-unit out of the first sub-library is able to bind to each sub-unit out of the second sub-library non-specifically. Although this method has led to the identification of unique antibodies against particular antigens, it fails to provide a method for identifying two partners of a specific binding pair when both are unknown.

A unique version of phage display which relies on non-infective phage has recently been proposed (Duenas, M. & Borrebaeck, C. A. K., Bio/Technology 12 (1994) 999-1002; EP 0 614 989). A version of this system that led to the identification of proteins from a cDNA library that interacts with the jun protein has been described (Gramatikoff et al., Nucleic. Acids Res. 22 (1994) 5761-5762). The same principle has been also shown to work with an antibody-antigen system (Krebber et al., FEBS Letters 377 (1995) 227-231).

In spite of the power of all the aforementioned genetic selection approaches, they are limited to the selection of interacting binding entities from only a single genetically-diverse population (library vs. individual).

It would, however, be highly desirable to simultaneously identify binding entities and their specific binding partners in a library vs. library setting, wherein preferably at least two genetically diverse populations are involved. A solution to this technical problem, i.e. the identification of interacting entities and the respective nucleic acid sequences from more than one genetically diverse population (library vs. library) is neither provided nor suggested by the prior art. The present invention solves the above technical problem by providing the embodiments characterized in the claims. By using these embodiments, it has become possible to increase exponentially the rate at which (poly)peptide-(poly)peptide interactions are detected. The present invention may find applications in the field of functional genomics, whereby different proteins of unknown functions can be related with other proteins.

Accordingly, the present invention relates to a method for identifying a plurality of nucleic acid sequences, said nucleic acid sequences each encoding a (poly)peptide capable of interacting with at least one further (poly)peptide encoded by a different member of said plurality of nucleic acid sequences, comprising the steps of:

(a)   providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides;

(b)   providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with further (poly)peptides as mentioned in step (a), wherein the vector molecules

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employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in step (a) and wherein at least one of said properties displayed by each of said vector molecules and/or the recombinant inserts used in steps (a) and (b), upon the interaction of a (poly)peptide from said first library with a (poly)peptide from said second library together generate a screenable or selectable property;

(c)   optionally, providing additional libraries of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with or causing interaction of (a) further (poly)peptide(s) as mentioned in step (a) and/or step (b), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in steps (a) and (b) and, optionally, at least one of said properties displayed by said vector molecule and/or the recombinant inserts used in step (c) together with at least one of said properties displayed by either said vector molecule and/or said recombinant insert used in steps (a) and/or (b), upon the interaction of a (poly)peptide from said additional library with either a (poly)peptide from said first library and/or a (poly)peptide from said second library generate a screenable or selectable property;

(d)   expressing members of said libraries of recombinant vectors or nucleic acid sequences mentioned in steps (a), (b) and optionally (c), in appropriate host cells so that at least one interaction is established;

(e)   selecting for the generation of said screenable or selectable property representing the interaction of said (poly)peptides;

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(f)   optionally, carrying out further selection, screening and/or purification steps; and

(g)   identifying said nucleic acid sequences encoding said (poly)peptides.

Thus, in the context of the present invention, the term "properties that are phenotypically distinguishable" relates alternatively to properties that are encoded by the vector molecule or to properties that are encoded by the recombinant insert or to both types of properties. As regards the vector-encoded properties, these may e.g. be resistance markers or requirements for special nutrients. It should be noted that the recombinant insert may comprise a nucleic acid portion encoding said property in addition to the nucleic acid portion responsible for the interaction.

In the context of the present invention, the term "different member " denotes a different entity which may be, but is not necessarily, structurally different.

Further, in the context of the present invention, the term "plurality" bears the meaning of "at least two".

The novel properties generated by the at least two recombinant inserts reflect the inventive principle of the present invention. That is, only if two (or more) (poly)peptides interact, for example, in a homo-dimeric or hetero-dimeric fashion, a screenable or selectable property is generated. The interaction between the two or more molecules may be a direct one or may be mediated indirectly. Examples for a direct interaction are the binding of an antibody encoded by a nucleic acid sequence from library 1 to a cDNA protein from library 2, the binding of a protein encoded by a nucleic acid sequence from cDNA library 1 to a protein from a cDNA library 2, as well as of an anti-idiotypic antibody encoded by a nucleic acid sequence from one of the libraries to a corresponding antibody encoded by a nucleic acid sequence from the other library. The nucleic acid sequences are preferably DNA and most preferably genes or parts thereof.

An example of an indirect interaction is the bridging of two (poly)peptides encoded by the two libraries which is mediated by a phosphorylating enzyme. Once the phosphorylation of one (poly)peptide encoded e.g. by library 1 is effected by the respective kinase, then this protein is capable of interacting with the second (poly)peptide encoded by library 2. The phosphorylating enzyme exemplifying this type of interaction may be encoded by a nucleic acid from (one of) the additional libraries and/or may be encoded by the genome of the host cell. Typically, the interaction of the two (poly)peptides forms a "bridge" of molecules, said "bridge" being detectable using an appropriate detection process. Conveniently, said bridge is detectable by a tag molecule that is associated with, encoded by or attached to one of the (poly)peptides encoded by library 1 or preferably 2.

Furthermore, the present invention relates to a method for identifying a plurality of nucleic acid sequences, said nucleic acid sequences each encoding a (poly)peptide capable of interacting with at least one further (poly)peptide encoded by a different member of said plurality of nucleic acid sequences, comprising the steps of:

(a)   expressing in appropriate host cells

(aa)   nucleic acid sequences contained in a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides;

(ab)   nucleic acid sequences contained in a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with further (poly)peptides as

mentioned in step (aa), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in step (aa) and wherein at least one of said properties displayed by each of said vector molecules and/or the recombinant inserts used in steps (aa) and (ab), upon the interaction of a (poly)peptide from said first library with a (poly)peptide from said second library together generate a screenable or selectable property;

(ac)   optionally, nucleic acid sequences contained in additional libraries of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with or causing interaction of (a) further (poly)peptide(s) as mentioned in step (aa) and/or step (ab), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in steps (aa) and (ab) and, optionally, at least one of said properties displayed by said vector molecule and/or the recombinant inserts used in step (ac) together with at least one of said properties displayed by either said vector molecule and/or said recombinant inserts used in steps (aa) and/or (ab), upon the interaction of a (poly)peptide from said additional library with either a (poly)peptide from said first library and/or a (poly)peptide from said second library generate a screenable or selectable property;

so that at least one interaction is established;

(b)   selecting for the generation of said screenable or selectable property representing the interaction of said (poly)peptides;

(c)   optionally, carrying out further screening, selection and/or purification steps; and

(d)   identifying said nucleic acid sequences encoding said (poly)peptides.

In a preferred embodiment of the method of the present invention, said screenable or selectable property is expressed extracellularly.

This embodiment is conveniently employed in a number of laboratories which would make use of rather conventional methodology of the extracellular detection of such properties, e.g. by column chromatography wherein the e.g. screenable tag is retained, in combination with e.g. plaque purification techniques, which allow the further purification of the cells that were originally enriched by e.g. the column chromatography step.

In a further preferred embodiment of the method of the present invention, said recombinant vector molecule in step (a)/(aa) (the step identified after the slash refers to the corresponding step of the second embodiment of the method of the invention identified hereinabove) gives rise to a replicable genetic package (RGP) displaying said (poly)peptides at its surface. In this context, the term replicable genetic package (RGP) refers to an entity, such as a virus or bacteriophage, which can be replicated following infection of a suitable host cell. In the case of bacteriophage, for example, the collection of nucleic acid sequences can be inserted into either a phage or phagemid vector in frame with a component of the phage coat, such as gene III, resulting in display of the encoded binding entities on the surface of the phage. Particularly preferred as a

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recombinant vector molecule is a recombinant phage, phagemid or virus, wherein said phage is most preferably

(a)   one of the class I phage fd, M13, If, Ike, ZJ/2, Ff;

(b)   one of the class II phage Xf, Pf1, and Pf3;

(c)   one of the lambdoid phages, lamda, 434, P1;

(d)   one of the class of enveloped phages, PRD1; or

(e)   one of the class paramyxo-viruses, orthomyxo-viruses, baculo-viruses, retro-viruses, reo-viruses and alpha-viruses.

In a further preferred embodiment of the method according to the invention, said selection step (e)/(b) is carried out by selecting polyphage comprising the interacting (poly)peptides. Polyphage contain more than one copy of phage genomic DNA. They occur naturally at a low to moderate frequency when a newly forming phage coat encapsulates two or more single-stranded DNA molecules. In the case of the present invention, the polyphage which are formed will contain at least two phage genomes, which may either (i) both be representatives of library 1, or (ii) both be representatives of library 2, or (iii) be representatives of each of library 1 and library 2, or (iv) be a combination of (i) to (iii) with at least one member of one of the additional libraries. The efficiency of polyphage production can be increased by the introduction of appropriate mutations into the phage genome, as is well known to those skilled in the art (see, for example, Lopez, J. and Webster, R.E., Virology 127 (1983), 177-193, Bauer, M. and Smith, G.P., Virology 167 (1988) 166-175, or Gailus, V. et al., Res. Microbiol. 145 (1994) 699-709).

In a further preferred embodiment of the method of the invention, said screenable or selectable property is connected to the infectivity of said RGP.

In this embodiment, use is made of the possibility that the infectivity of e.g. a bacteriophage can be manipulated, said infectivity being directly correlated with the interaction of said (poly)peptides.

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In a most preferred embodiment of the method of the present invention, said RGP is encoded by said recombinant vector used in step (a)/(aa) and rendered non-infective and infectivity of said RGP is restored by interaction of said (poly)peptide of step (a)/(aa) with the (poly)peptide of step (b)/(ab) and/or (c)/(ac), said (poly)peptide of step (b)/(ab) and/or (c)/(ac) being fused to a domain that confers infectivity to said RGP.

In a further most preferred embodiment of the method of the invention, said RGP is rendered non-infective by modification of a genetic sequence which encodes a surface protein necessary for the RGP's binding to and infection of a host cell.

These preferred and most preferred embodiments of the method of the present invention relating to the infectivity of the RGP serve as an alternative to the use of the screenable tag. In these embodiments, advantage can be taken of the phenomenon of selective infection (Krebber et al., FEBS Letters 377 (1995) 227-239). While the screenable tag enables physical separation of molecules from others in the population, the use of selective infection enables positive selection for the interacting pair. This phenomenon relies on the use of a construct which can selectively restore infectivity to phage which have been rendered non-infective by, for example, deletion of all but the C-terminus of the gene III protein. Use of such phage for displaying library 1 gives non-infectious phage carrying the binding entity. Co-expression with library 2 allows interactions between binding entities and binding partners to be established, as described above. Although the phage which carry the binding entity-binding partner pair are non-infective, infectivity can be restored if, in place of the screenable tag referred to above, an infectivity protein is used. In this context, the term infectivity protein refers to a substance which, when associated with the phage, can enable it to penetrate a bacterial host, where it is subsequently replicated. An example of an infectivity protein is the N-terminus (at least the first 220 amino acids) of gene III protein of the filamentous bacteriophage.

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The infectivity protein confers on those phage which carry it, the ability to be replicated. Thus, only those phage which carry the binding entity/partner pair are replicated. Purification of hybrid phage containing genes from both libraries 1 and 2 then relies e.g. on the use of two selectable markers as indicated above. The genes in the phage can then be identified using methodology well known to those skilled in the art.

An additional preferred embodiment of the present invention relates to a method, wherein said recombinant vector molecules in step (a)/(aa) give rise to a fusion protein which is expressed on the surface of a cell, preferably a bacterium.

These fusion proteins, upon interaction with a suitable binding partner from library 2 connected e.g. with a screenable tag can be detected on the surface of host cells which may be, for example, bacteria, yeast, insect cells or mammalian cells. The display of fusion proteins on bacterial surfaces per se is well known in the art. Thus, lipoproteins (Lpp), outer membrane proteins A (OmpA), and flagella have been used to target antibodies and peptides to the cell surface of E.coli. Fuchs et al., Bio/Technology 9 (1991) 1369-1372, WO93/01287, presented a single chain antibody on the surface of E.coli as a fusion protein with the N-terminus of the peptidoglycan-associated lipoprotein. The antibody was visualized by the binding of fluorescently labeled antigen and fluorescently labeled antibodies directed to the linker peptide of the displayed single chain antibody. Francisco et al., Proc. Natl. Acad. Sci. USA 90 (1993) 10444-10448, and Georgiu, G. et al., WO93/10214, displayed antibodies on the E.coli surface by fusing the N-terminus of a single chain antibody to the C-terminus of OmpA while the N-terminus of OmpA was fused to the signal sequence and the first nine amino acids of Lpp. Binding of a fluorescently labeled antigen to the OmpA-antibody fusion protein was detected by FACS. Klauser (WO 95/17509) transferred the IgA protease system from Neisseria to E.coli to facilitate display of antibodies. Integration of the beta-domain of the IgA protease precursor into the outer membrane lead to the transport of the

protease domain across the membrane followed by autoproteolytic release into the medium. Antibodies linked to the beta-domain of IgA protease are therefore presented on the surface of bacteria. Further, Lu, Z. et al., Bio/Technology 13 (1994) 366-371, described a system for displaying peptides on the surface of the bacterium by fusing it to thioredoxin and the bacterial flagella, to screen for peptide mimics of the epitope for an anti-IL-8 antibody.

The further identification of the desired nucleic acid molecule encoding the interacting (poly)peptides may then be effected by methods known in the art, e.g. by purifying host cells displaying a tag on their surface and further by antibioticum-based selection techniques, DNA purification and sequencing.

In a particularly preferred embodiment of the method of the present invention, said bacterium is Neisseria gonorrhoe or E.coli and said fusion protein consists of at least a part of a flagellum, Iam B, peptidoglycan-associated lipoprotein or the Omp A protein and said (poly)peptide.

As has been repeatedly pointed out hereinabove, a tag connected to the (poly)peptide encoded by library 2 can conveniently be used in the identification strategy of the desired nucleic acid sequences. Accordingly, in a further preferred embodiment of the method of the invention, said (poly)peptides encoded by said recombinant vector molecules of step (b)/(ab) or (c)/(ac) are linked to at least one screenable or selectable tag. In this context, the term screenable or selectable tag refers to a short sequence of amino acids which can be recognized and bound by a particular substance. Tags are commonly used for the purification of biomolecules: examples are His(n), where n = 4-6 which can be bound either by Ni, or a specific antibody, and the flag and myc tags which are recognized by appropriate antibodies. In either of these cases, the tag can be encoded as a C-terminal fusion to all binding partners in library 2. In accordance with the present invention, the tag can be used to isolate e.g. the polyphage referred to

above. Thus, the interaction between the phage-bound binding entity, and its interacting binding partner, establishes a connection between the phage particle and the screenable or selectable tag. This feature can be exploited in a step which relies on e.g. affinity chromatography to isolate the polyphage carrying the interacting molecules. In a final step, those polyphage which carry two distinct nucleic acid molecules and preferably genes (encoding binding entity and binding partner) can be separated from those carrying only one of the two genes e.g. by selection based on transduction or different selectable markers (e.g. antibiotic resistance) present in the individual genomes. In this way, the genes which encode the two interacting molecules can be identified.

A most preferred embodiment of the present invention relates to a method wherein said screenable or selectable tag is encoded by said recombinant vector of step (b)/(ab) or (c)/(ac).

A further most preferred embodiment of the present invention relates to a method wherein said screenable or selectable tag is selected from the list His(n), myc, FLAG, malE, thioredoxin, GST, streptavidin, beta-galactosidase, alkaline phosphatase T7 gene 10, Strep-tag and calmodulin. These screenable tags are all well known in the art and are fully available to the person skilled in the art.

In an additional particularly preferred embodiment of the method of the invention, said screenable or selectable tag is encoded by the genome of the host cell.
An example for this embodiment is an anti-Fc-receptor specific antibody that is expressed by the host cell and could function as an additional bridge in e.g. purification by column chromatography. Another example of this embodiment is an enzyme produced by the host cell that creates a tag such as a phosphorylation on (poly)peptides of the second library without destroying the interaction of (poly)peptides of step (b)/(ab)

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with (a)/(aa) so that the modification caused by the enzyme is now the screenable or selectable tag.

In a further preferred embodiment of the method of the invention, said (poly)peptides encoded by the nucleic acid sequences of said additional libraries of step (c)/(ac) cause the interaction of said (poly)peptides of steps (a)/(aa) and (b)/(ab) via phosphorylation, glycosylation, methylation, lipidation or farnesylation of at least one of said (poly)peptides of steps (a)/(aa) and (b)/(ab).

An additional preferred embodiment of the invention relates to a method wherein said host cells in step (d)/(a) are spatially addressable, and the nucleic acid sequences mentioned in step (g)/(d) are retrieved from the corresponding spatially addressable host cell.

In the context of the present invention, the term "spatially addressable" refers to a situation where the individual cells harboring one of the potential combinations of members of the first, second and optionally additional libraries are identifiable by their relative position, e.g. by their position on a master plate. The screening or selection may, for example, be performed either with single clones derived from the master plate, or on a replica plate, thus maintaining the connection between the screenable or selectable property and the information contained in the host cell on the master plate.

An additional preferred embodiment of the invention relates to a method wherein said screenable or selectable property is expressed intracellularly.

Particularly preferred is a method wherein said screenable property is the transactivation of the transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu or resistance genes

giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline, or streptomycin.

Furthermore, use can be made of the yeast 2-hybrid system referred to hereinabove or the interaction trap system (Brent et al., EP-A 0 672 131) or of a prokaryotic version analogous to the above recited systems, utilizing the toxR system of Vibrio cholerae (Fritz, H.-J. et al., EP-A 0 630 968). It is within the skills of the person skilled in the art to combine further screening systems known in the art with the method of the present invention.

In a further preferred method of the present invention, said recombinant vectors of step (a)/(aa), (b)/(ab) and (c)/(ac) comprise recombination promoting sites and in said step (e)/(b) recombination events are selected for, wherein said nucleic sequences encoding said (poly)peptides of step (a)/(aa), said nucleic acid sequences encoding said (poly)peptides of step (b)/(ab) and optionally said nucleic acid sequences encoding said (poly)peptides of step (c)/(ac) are contained in the same vector. In this approach, the two genes can be coupled in a single vector, and packaged in a phage of standard size, if appropriate recombination sites are incorporated in the vectors carrying libraries 1 and 2. Again, the phage which carry both nucleic acid sequences and genes are purified with the use of e.g. the screenable tag. If recombination is used to couple the genes from the two libraries, some of the hybrid progeny phage will contain nonrecombinant genomes, since site-specific recombination is not very efficient. However, the hybrid phage can be selected by re-infection of host cells that do not contain library 2 followed by another round of selection of the screenable tag.

In a particularly preferred embodiment of the method of the invention, said recombination events are mediated by the site-specific recombination mechanisms Cre-lox, attP-attB, Mu gin or yeast flp.

In a further particularly preferred embodiment of the method of the invention, said recombination promoting sites are restriction enzyme recognition sites and said recombination event is achieved by cutting the recombinant vector molecules mentioned in steps (a)/(aa), (b)/(ab) and optionally (c)/(ac) with at least two different restriction enzymes and effecting recombination of the nucleic acid sequences contained in said vectors by ligation.

The invention relates in an additional preferred embodiment to a method wherein said identification of said nucleic acid sequences is effected after the selection step (e)/(b) via PCR and preferably sequencing of said nucleic acid sequences after said PCR.

After said selection step (e)/(b), PCR can be carried out with the enriched desired product, conveniently using primers that hybridize to the vector portion of the recombinant vector molecule. Sequencing of the PCR-product may then be carried out according to conventional methods.

In a further preferred embodiment of the method according to the invention, said recombinant vectors of step (a)/(aa), (b)/(ab) and/or (c)/(ac) comprise at least one gene encoding a selection marker.

Said genes encoding said selection markers are preferably different in each of the vectors of step (a)/(aa), (b)/(ab) and/or (c)/(ac), i.e. said vectors comprise genes encoding different selection markers. Said selection markers can conveniently be used for the further purification envisaged in step (f)/(c). For example, a polyphage comprising two members of each library 1 and 2 can be selected for on the basis of a double resistance to antibiotics. Also, a successful recombination event may create a new recombinant vector carrying both nucleic acid molecules from library 1 and 2 as well as genes encoding different selection markers. Again, the selection for a twofold resistance will assist in the identification of the desired product.

In a particularly preferred embodiment of said method, said selection marker is a resistance to an antibiotic, preferably to ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

A further preferred embodiment of the present invention relates to a method wherein said host cells are F' and preferably E.coli XL-1 Blue, K91 or its derivatives, TG1, XL1kan or TOP10F.

In a particularly preferred embodiment of the present invention, said RGPs are produced with the use of helper phage taken from the list R408, M13k07 and VCSM13, M13de13, fCA55 and fKN16 or derivatives thereof.

Further preferred is a method wherein at least one of said genetically diverse nucleic acid sequences encode members of the immunoglobulin superfamily.

Said method is particularly preferred, if said genetically diverse nucleic acid sequences encode a repertoire of immunoglobulin heavy or light chains.

In an additional preferred embodiment of the present invention, in said method said genetically diverse nucleic acid sequences are generated by a mutagenesis method. Various mutagenesis methods are well known to the person skilled in the art and need not be described in here in any further detail.

The present invention relates in an additional preferred embodiment to a method in which said genetically diverse nucleic acid sequences are generated from a cDNA library.

In a final preferred embodiment of the method of the invention, said nucleic acid sequences are genes or parts thereof.

As used herein, the term "parts thereof" relates to parts of genes that encode a product that is capable of interacting with a product encoded by any of the other libraries. Thus, it is well known that various proteins are comprised of different domains. Only one of said domains may be capable of interacting with a different (poly)peptide. Such a domain might be encoded by a part of said gene in accordance with the present invention.

The invention also provides for identifying genes encoding more than two interacting peptides or proteins. This can be achieved by using additional vectors encoding genetically diverse additional nucleic acids by an extension of the method described above. As previously, the presence of either a screenable tag or an infectivity protein is used to purify phage carrying genes which encode the components of the complex. Again, the genes in the phage can then be sequenced using methodology well known to those skilled in the art.

Additionally, the present invention relates to a kit comprising at least

(a)   a recombinant vector molecule as described in step (a)/(aa) or a corresponding vector molecule;

(b)   a recombinant vector molecule as described in step (b)/(ab) or a corresponding vector molecule; and, optionally,

(c)   at least one further recombinant vector molecule as described in step (c)/(ac) or a corresponding vector molecule.

As a rule, if recombinant vector molecules are comprised in said kit, they will comprise a library of nucleic acid molecules. In other words, the kit of the invention will contain a plurality of different recombinant vector molecules.

Legends to Figures and Tables

Figure 1: General description of the polyphage principle

a) transform to E. coli hosts

b) infect host containing library1 with helper-phage to package library1 into phage

c) infect cells containing library2 with phages containing library1 leading to cells harboring members of library1 and library2; the presence of library1 and library2 is selected by the presence of the 2 antibiotic resistance markers

d) expression of library1 and library2-tag gene products

e) infect cells with engineered helper-phage to induce polyphage production

Note 1: Polyphage does not discriminate which genome to package therefore the possibilities resulting from step e) arise in an infected cell. To select for the polyphage containing the right packaged genomes the subsequent step is required

f) select for tag e.g., infectivity-mediating protein, in which case ability to infect is selected and

g) select for ability to confer resistance to 2 antibiotics to infected cells

Note 2: Only polyphages that satisfy f) + g) represent phages that display the correct interacting pair and the corresponding genetic information

Figure 2: Co-transformation of two phagemids, polyphage formation and selection via His-tag: general description

A, B: libraries of phagemids, preferably with different resistance markers; A: fusions to gIIIp; B: fusions to tag (His); after co-transformation phage production leading to a phage population displaying cognate pairs (left part of the Figure) or not (right part), after selection infection of host cells, selection for double-resistance

Alternative methods include the infection of cells harbouring a plasmid- or phagemid-based library B with a phage library A (prerequisite again: interference-resistant constructs).

Figure 3: pBS vector series: functional map and sequence of pBS13

Figure 4: Co-existence of phagemids: results of restriction digest

Restriction analysis of clones of double resistances (Amp/Cm). R1: pIG10.3, *Xba/ScaI*; R2: pBS13, *Xba/ScaI*, R1+R2: R1 and R2 are mixed in approx. equal proportion; M1: marker λ: *BstEll*; M2: marker pBR322: *MspI*; 1 to 10: randomly picked clones: *Xba/ScaI*

Figure 5: Phagemid vector pYING1-C1: functional map

containing the fos peptide. The corresponding vectors pYING1-C2 and pYING1-C3 contain instead of fos the p75 and the IL16 peptides, respectively

Figure 6: Phagemid vector pYANG3-A: functional map

containing the jun peptide. The corresponding vectors pYANG3-Ape2, pYANG3-Ape3, and pYANG3-Ape10 contain instead of jun the p75-binding peptides pe2, pe3, and pe10, respectively

Figure 7: Analysis of selected clones (see Table 2):

7.a: Restriction digest of clones before and after selection

R: pYANG3-Ape2: *XbaI*; M1: marker λ: *BstEll*; M2: marker pBR322: *MspI*; α/1 to 10: randomly picked clones before selection: *XbaI/HindIII*; β/1 to 10: randomly picked clones after selection: *XbaI/HindIII*; size expected: jun-gIII: 745 bp; fos: 256 bp; p75: 577 bp; IL-16: 502 bp

7.b: PCR reaction of clones after selection with primers OPEP5L and OGIII3

R1: pYANG3-A as template; R2: pYANG3-Ape2 as template; M: marker λ: *BstEll*; β/1 to 10: randomly picked clones after selection as templates

Figure 8: Phagemid vector pING1-C1: functional map

containing the His-tag peptide. The corresponding vector pING3-C1 contains an additional FLAG epitope; pING1-C2 and pING3-C2 contain

the Strep-tag instead of His-tag, with pING3-C2 containing an additional FLAG epitope.

Figure 9: Phagemid vector pONG3-A: functional map

for the generation of phage-display libraries (gIII fusions)

Figure 10: Co-transformation of phage and plasmid, polyphage formation and selection *via* SIP: general description

fA: library A in phage construct; B: library B, library members fused to IMP; preferably different resistance markers on phage and plasmid; after co-transformation production of phages; in the case of cognate-pair interaction formation of infectious phages; selection; by plating on double-resistance identification of polyphage particles.

Figure 11: Phage vector fhag1A: functional map

for phage-display of the α-HAG scFv

Figure 11a: CAT gene module: functional map and sequence

Figure 12: Phage vector fjun1A: functional map

for phage-display of the jun peptide

Figure 13: Phage vector fjun1B: functional map

for phage-display of the jun peptide

Figure 14: Phage vector fpep3_1B: functional map

for phage-display of the peptide pe3 binding to the intracellular domain of p75

Figure 15: Phage vector fNGF_1B: functional map

for phage-display of NGF

Figure 16: Plasmid pUC19/IMPhag: functional map

containing fusion of HAG peptide to the N-terminal domains of gIIIp (IMP)

Figure 17: Plasmid pUC18/IMPp75: functional map

containing fusion of the intracellular domain of p75 to the N-terminal domains of gIIIp (IMP); pUC18/IMPfos contains the fos peptide instead of the intracellular domain of p75

Figure 18: Plasmid pUC18/IMPIL16: functional map

containing fusion of IL16 to the N-terminal domains of gIIIp (IMP)

Figure 19: Analysis of selected clones (see Table 3)

Lane 1: marker λ: *Bst*EII; lanes 2 to 20: polyphage transductant clones #1

to #19 digested with *Xba/Hind*III; f.._1b: fragment of phage vector after

digest; pUC18: fragment of plasmid after digest; α-HAG: fragment

containing anti-HAG scFv fused to gIIIc; IMP-p75 and IMP-HAG: fragment

containing IMP fused to p75, and IMP-HAG peptide, respectively; pep3-

gIIIs: fragment containing pep3 fused to gIIIc (s: short version)

Figure 20: Co-transformation of phagemids, *in vivo* recombination and selection *via*

His-tag: general description

A, B: libraries of phagemids; preferably with different resistance markers;

A: fusions to gIIIp; B: fusions to tag (His); both constructs containing

recombination-promoting sites (*) such as lox/loxP; after co-transformation

and recombination production of phages; selection *via* Ni-NTA; re-infection

of host cells, selection for double-resistance

Figure 21: *In vitro* recombination and selection *via* His-tag: general description

A, B: libraries of phagemids; preferably with different resistance markers;

A: fusions to gIIIp; B: fusions to tag (His); both constructs containing

corresponding recognition sites for restriction enzymes (+/o); after digest

and co-ligation transformation and production of phages; selection *via* Ni-

NTA; re-infection of host cells, selection for double-resistance

Figure 22: Phage vector fjunhag: functional map for phage display of the jun peptide

Figure 23: Spatial *in vivo* SIP: general description

After transformation or co-transformation according to any of the methods

described above, a master plate is made. From that phages secreted from

individual clones can be analyzed individually (top), or a replica (migration

of secreted phages through filter disc) can be made whereon selection for

the presence of a tag or infectivity can be performed. By going back to the

master-plate, the information for selected cognate interacting pairs can be retrieved without requiring recombination and/or polyphage production.

Figure 24: *E. coli* display: general description

A, B: libraries of phagemids; preferably with different resistance markers; A: fusions to E.coli surface-display protein; B: fusions to tag (His); after co-transformation expression of constructs; surface-display; in the case of cognate interaction taking place, display of tag on the surface of the host cell; selection

Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence

Table 1: Phagemids constructed for Experiments 2 and 3

Table 2: Results of Experiment 2 (see Figure 7)

2.a: Combination of phagemids present in initial library (α)

2.b: Combination of phagemids present after selection (β)

Table 3: Results of Experiment 4 (see Figure 19)

3.a: Identification of phage/plasmid present in individual clones

3.b: Test for infectivity of individual clones

The examples illustrate the invention.

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Example 1: General description of the polyphage principle (Figure 1)

The binding entities which comprise library 1 may be peptides or proteins, and are encoded by a genetically diverse collection of first nucleic acid sequences. These nucleic acid sequences are inserted into a first vector which allows for display of the encoded binding entities on the surface of a replicable genetic package. For the purposes of subsequent selection, the first vector should also carry a gene encoding a selectable marker, such as an antibiotic resistance. The binding partners which comprise library 2 may be peptides or proteins, and are encoded by a genetically diverse collection of second nucleic acid sequences which are inserted into a second vector. By way of example, this second vector may be a plasmid, or even a phage or phagemid, in which case the origin of replication should be distinct from that of the first vector. For the purposes of subsequent selection, the second vector should also carry a gene encoding a selectable marker, such as an antibiotic resistance, preferably distinct from that present in the first vector. To facilitate purification of the complex to be formed between any binding entity-binding partner pair, a screenable tag can be conveniently attached to members of library 2.

The two genetically diverse collections of nucleic acids are then introduced into a population of host cells in such a way that encoded libraries 1 and 2 can be expressed. This can be achieved by either (i) co-transformation of the two vectors, or, as actually shown in the figure, (ii) packaging one of the collections of nucleic acids into a vector (such as a bacteriophage) which can be used to infect with high efficiency a population of cells into which the complementary collection of nucleic acid has been introduced. The result is a population of cells in which individual cells carry representatives of each library.

Expression of the two collections of nucleic acids results in the production of pairs of molecules, one from each library, in the host cells. In each case, one or more members

of the library of binding entities is incorporated into the coat of an RGP. In some cells, an interaction will be established between a binding partner on the surface of the RGP and a binding partner expressed from library 2. When such an interaction is established, the RGP therefore carries both the binding entity and the binding partner.

The RGPs displaying such an interaction can then be further purified with the help of polyphage and differing selection markers, as has been discussed hereinabove. After such selection, the nucleic acid sequences encoding one or both binding partners can be conveniently identified by methodology known in the art, such as DNA sequencing.

Example 2: Co-transformation of phagemids with same *E. coli* origin of replication, polyphage formation, and selection of correct pairing interactions *via* His-tag

2.1: Principle (see Figure 2)

To demonstrate that polyphage formation allows the retrieval of the genetic information for cognate protein pairs selected using a tag fused to one member of the protein pair, two separate, small libraries in phagemid vectors are constructed.

2.2: Test of co-existence of phagemids with the same *E. coli* origin of replication:

Prerequisite for the formation of polyphage particles containing two different phagemids is that the different phagemid vectors can co-exist in the host cell.

The vector pBS13 is a derivative of the vector (Krebber *et al.*, 1996) containing a chloramphenicol-resistance gene instead of the kanamycin-resistance gene and a beta-lactamase gene cassette instead of the 2H10-gIII fusion gene, and can be assembled by standard methods starting from pto2H10a3s. Figure 3 contains the functional map and the sequence of pBS13. pIGHAG1A (see Example 4.2.1.f) is digested with *Xba*I and *Hind*III. The 1.3 kb fragment containing the anti-HAG gene fused with the C-

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terminal domain of filamentous phage pIII protein is isolated and ligated with a pre-digested phagemid vectors pIG10.3, and pBS13 (XbaI-HindIII) to create the vectors pIG10.3-scFv(anti-HAG) (Ap^R) and pBS13-scFv(anti-HAG) (Cm^R), respectively. The vectors are used to transform competent XL-1 Blue cells and selected on LB plates containing Amp/Cm/Tet and glucose (20 mM).

The phagemids from clones of double-resistant colonies (Amp/Cm) are isolated. The restriction digestions indicate the co-isolation of both phagemids from the single colonies (Figure 4).

2.3: Design of libraries A and B:

Library A contains three cyclic peptides each binding to the intracellular domain of the low affinity nerve growth factor (NGF) receptor (see Example 4), and a leucine zipper domain derived from the jun transcription factor, all N-terminally fused to the C-terminal domain of gIII from filamentous phage.

Library B encodes 3 members, namely the leucine zipper domain of the fos transcription factor which heterodimerizes with jun *via* this domain, the intracellular domain of the NGF receptor p75, and, as a negative control which does not interact with library A members, IL-16, all fused at the N-terminus with a His$_6$-peptide as tag (Hochuli *et al.*, 1988; Lindner *et al.*, 1992).

The cognate pairings are from the interaction between jun and fos (Crameri and Suter, 1993), and p75 and selected cyclic peptides (see Example 4). A non-cognate pairing would occur among the non-cognate pairs mentioned and among jun, or one of the cyclic peptides, and IL-16.

2.4: PCR amplification of the individual constructs

Fos, N-terminus fused to His$_6$, is PCR amplified using pOK1 (Gramatikoff *et al.*, 1994) as template and oligonucleotides OFOS-5 and OFOS-3 as primers, where His$_6$ is

encoded in the OFOS-5 primer. Jun is PCR amplified using pOK1 as template and oligonucleotides OJUN-5 and OJUN-3 as primers.

OFOS-5   5'- GGGG*GATATC*CACCACCACCACCACCACCTGCGGTGGTCTGACC

OFOS-3   5'- GGGG*GAATTC*CAACCACCGTGTGCCG

OJUN-5   5'- GGGG*GATATC*GGTGGTCGGATCGCC

OJUN-3   5'- GGGG*GAATTC*ACCACCGTGGTTCATGAC

The hot-start procedure is used. A step-wise touch-down PCR is applied: 92°C, 1 min; 58-52°C, $\Delta T = 2$°C, 1 min; 72°C, 1 min. This is followed by 26 cycles (92°C, 1 min; 52°C, 1 min; 72°C, 1 min).

The PCR products are purified using QIAquick kit (Qiagen) and eluted in ddH$_2$O. They are then overnight digested with *EcoRI* and *EcoRV*.

The p75 fragment is also PCR amplified using pUC18-IMPp75 (see Example 4) as template and oligonucleotides OP75-5 (where His$_6$ is encoded) and OP75-3 as primers:

OP75-5   5'- GGGG*GATATC*CACCACCACCACCACCACAAGAGGTGGAACAGC

OP75-3   5'- GGGG*GAATTC*CACTGGGGATGTGGCAG

The same PCR and restriction digestion conditions as above are applied.

The IL-16 fragment is amplified from the cDNA clone **pcDNA3-ILHu1** (M. Baier, Paul Ehrlich Institute, Germany; Baier *et al.*, 1995; Bannert *et al.*, 1996), using OIL16-5 (where His$_6$ is encoded) and OIL16-3 as primers.

OIL16-5 5'- GGGG*GATATC*CACCACCACCACCACCACCCCGACCTCAACTCCTC

OIL16-3 5'- GGGG*GAATTC*GGAGTCTCCAGCAGCTG

The same PCR and restriction digestion conditions as above are applied.

In all cases, the fragments are readily amplified and digested.

2.5: Cloning into intermediate vectors

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The digested PCR fragments are gel-purified (QIAquick kit, Qiagen) and eluted into TE buffer. The *EcoRV/EcoRI* fragment of pIG1 vector (Ge *et al.,* 1995) is also isolated. The digested PCR fragments of fos, p75, and IL-16 are ligated into the vector fragment, and the ligated vectors transformed into TG1 cells.

The constructs in the pIG1 vector contains the OmpA signal sequence fused in-frame with the constructs.

The correct clones are screened and confirmed by sequencing. They are then *Xbal/Hind*III digested, and the fragments are isolated.

2.6: Cloning into the expression vectors

The isolated fragments from 2.3 are inserted into pBS13 also excised with *Xbal/Hind*III, resulting in vectors **pYING1-C1** (Fos), **pYING1-C2** (p75), **pYING1-C3** (IL-16) (see Figure 5). The fragment containing jun is cloned into pIG10.3 vector *via EcoRV/EcoRI* resulting in **pYANG3-A** (see Figure 6). The anti-p75 peptides pe2, pe3 and pe10 (see Example 4) are cloned into pIG10.3 via *Xbal/Hind*III, resulting in vectors **pYANG3-Ape2, -Ape3** and **-Ape10**, respectively (see Figure 6).

2.7: Selection of correct pairing *via* His-tag

TG1 cells are transformed with the combination of pYANG3-A + pYING1-C1, or pYANG3-A + pYING1-C2, or pYANG3-A + pYING1-C3, or (pYANG3-Ape2, -Ape3 and -Ape10) + pYING1-C1, or (pYANG3-Ape2, -Ape3 and -Ape10) + pYING1-C2, or (pYANG3-Ape2, -Ape3 and -Ape10) + pYING1-C3, thus creating all possible combinations separately to ensure the presence of each of them in the selection experiment. The transformed cells are plated on ampicillin/chloramphenicol-containing LB agar plates, and colonies with double resistance (Ap^R/Cm^R) are selected.

The colonies are scraped off the plates and used to inoculate 2xYT medium (Amp/Cm) and shaken at 37°C for 3 hrs. The cultures are induced (1 mM IPTG) at 30°C for 1 hr and infected with R408 (Stratagene) at 37°C for 30 min. The cultures are shaken at RT for 3 hrs, kanamycin is added and shaking continued at RT overnight.

The phage particles are harvested from the overnight cultures, mixed and PEG-precipitated. The phages are directly selected on immobilized Ni-NTA (NI-NTA HisSorb Strips, Qiagen). The eluted phages are used to infect TG1 cells, which are plated on ampicillin/chloramphenicol-containing LB agar plates, and colonies with double resistance (Ap^R/Cm^R) are selected.

The phagemids of selected clones are isolated and analyzed by restriction digest (see Figure 7.a) and used as templates for PCR screening. Primer OPEP5L is used to amplify the pYANG3-Ape2, -Ape3 and -Ape10 constructs specifically (see Figure 7.b).

OPEP5L   5'- GACTACAAAGATGTCGACTG

There is a specific enrichment of constructs of correct pairing (Table 2).

Example 3: Interactive screening of *E. coli* genomic DNA libraries (Polyphage/tag system)

3.1: Principle (see Figure 2)

Instead of using two model libraries as in Example 2, a genomic DNA library of *E. coli* is prepared to be screened against itself to identify interacting *E. coli* peptides or proteins.

3.2: Construction of display and expression vectors for genomic DNA

Expression vectors are constructed having a blunt-end restriction site SmaI inserted either in front of His-tag, Strep-tag (Schmidt and Skerra, 1994) or the C-terminal domain of gIII (gIIIc) *via* oligonucleotide cassettes or PCR.

The self-complementary oligonucleotides OHIS5 & OHIS3, and OSTREP5 & OSTREP3, are used to create ds DNA cassettes encoding the His-tag, and the Strep-tag, respectively.

OHIS5   5'- AATTCCCCGGGCACCACCACCACCACCACTGATA

OHIS3   5'- AGCTTATCAGTGGTGGTGGTGGTGGTGCCCGGGG

OSTREP5   5'-   AATTCCCCGGGTCTGCTTGGCGTCACCCGCAGTTCGGTGGT-
                    TGATA

OSTREP3   5'-   AGCTTATCAACCACCGAACTGCGGGTGACGCCAAGCAGACC-
                    CGGGG

The cassettes upon phosphorylation and annealing recreate the *EcoR*I and *Hind*III sites.
The cassettes are inserted into pIG1 and pIG3 vectors (Ge *et al.*, 1995) cut by the same
restriction enzymes. The resulting vectors are pING1-A1, pING3-A1 (for His tag in pIG1
and pIG3 vectors) and pING1-A2, pING3-A2 (for Strep-tag), respectively. The correct
vectors are screened for the presence of *Xma*I site (isoschizomer of *Sma*I) and the
constructs are confirmed by sequencing. The *Xba*I/*Hind*III fragments of these vectors
are inserted into pBS13 vector, linearized with the same enzymes, resulting in vectors
pING1-C1, pING3-C1 and **pING1-C2, pING3-C2,** respectively (see Figure 8).

The gIIIc fragment containing the *Sma*I site is generated from PCR amplification of
pIG10.3 vector using primers OGIII5 and OGIII3, where OGIII3 anneals 3' of the gene III
in the vector:

OGIII5   5'- CG*GAATTC*CCCGGGGAGCAGAAGCTGATC

OGIII3   5'- TTTTTCACTTCACAGGTC

Three rounds of PCR are performed with a hot-start: 92°C, 1 min; 46°C, 1 min; 72°C, 1.5
min. This is followed by 30 rounds of: 92°C, 1 min; 50°C, 1 min; 72°C, 1.5 min.

The PCR product is purified (QIAquick) and digested with *EcoR*I and *Hind*III. The
fragment is gel-purified (QIAquick) and ligated into pIG10.3. The sequence of the
resulting vector, **pONG3-A** (see Figure 8), is confirmed by restriction analysis and by
sequencing.

3.2: Selection of Interacting Pairs from *E. coli* Genomic DNA via His-tag

Genomic DNA of *E. coli* strain XL-1 Blue (Stratagene) is isolated using the Blood & Cell
Culture DNA Maxi kit (Qiagen) and eluted in TE buffer (pH 8.0). 200 µg of the DNA is

taken and sonicated (50 cycles, 270 mA, 0.5 s/stroke). The fragmented DNA (average size: max. 0.7 kB) is blunt-ended by a fill-in reaction with T4 DNA polymerase.

Vectors pING1-C1 and pONG3-A are digested with EcoRV and Smal, the vector fragments are gel-purified (Qiagen). The vector fragments are then ligated with the blunt-ended genomic DNA at 16°C overnight. The ligation mixtures are taken to transform TG1 cells.

The pING1-C1 and pONG3-A transformants are scratched from the plate and used to inoculate 2xYT medium containing Cm/glucose or Amp/glucose, respectively. The pING1-C1 culture is infected with helper-phage (VCSM13 or M13k07) and phage particles are isolated. These phage particles are used to infect log-phase cells containing the pONG3-A library. The resulting culture is plated out on large Amp/Cm/glucose plates.

The colonies are scratched from the surface of the plates above and transferred to 2xYT medium containing Amp/Cm. After 30 min shaking at 37°C, the culture is then induced (1 mM IPTG) for 30 min, infected with helper-phage at 37°C for 30 min and shaken at RT overnight.

The phage particles are harvested from the overnight culture and PEG-precipitated. They are selected on immobilized Ni-NTA (NI-NTA HisSorb Strips, Qiagen). The eluted phages are used to infect log-phase TG1 cells. Selected protein pairs are characterised by determination of their corresponding DNA sequences.

Example 4: Polyphages and Selection of Correct Pairing Interactions via SIP

4.1: Principle (see Figure 10)

The purpose of this experiment is to show that from a combination of 2 libraries one can isolate and identify the correct interacting pairs using the SIP (Selectively Infective Phage: Krebber et al., 1995; the term "IMP" used in the experimental section denotes "Infectivity mediating particle" comprising the N-terminal domains of the gene III protein

of filamentous phage) selection system, and recover the information about both interacting partners *via* the formation and selection of polyphage particles. The library members forming interacting pairs with members of the corresponding library are being 'doped' with library members that do not interact with members of the corresponding library, and thus should not give a positive SIP selection.

4.2: Construction of vectors

4.2.1: fhag1A (see Figure 11)

a. The phage vector f17/9-hag (Krebber *et al.*, 1995) is digested with EcoRV and XmnI. The 1.1 kb fragment containing the anti-HAG Ab gene is isolated by agarose gel electrophoresis and purified with a Qiagen gel extraction kit. This fragment is ligated into a pre-digested pIG10.3 vector (EcoRV-XmnI). Ligated DNA is transformed into DH5a cells and positive clones are verified by restriction analysis. The recombinant clone is called **pIGhag1A**. All cloning described above and subsequently are according to standard protocols (Sambrook *et al.*, 1989)

b. The vector f17/9-hag (Krebber *et al.*, 1995) is digested with EcoRV and StuI. The 7.9 kb fragment is isolated and self-ligated to form the vector **fhag2**.

c. The chloramphenicol resistance gene (CAT) assembled *via* assembly PCR (Ge and Rudolph, 1997) using the the template pACYC (Cardoso and Schwarz, 1992) (Figure 11a shows the functional map and the sequence of the CAT gene) is amplified by the polymerase chain reaction (PCR) with the primers:

CAT_BspEI(for):   5' GAATGCTCATCCGGAGTTC

CAT_Bsu36I(rev): 5' TTTCACTGGCCTCAGGCTAGCACCAGGCGTTTAAG

d. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with BspEI and Bsu36I then ligated into pre-digested fhag2 vector (BspEI-Bsu36I; 7.2 kb fragment) to form **fhag2C**.

e. The vector fhag2C is digested with EcoRI and the ends made blunt by filling-in with Klenow fragment. The flushed vector is self-ligated to form vector **fhag2CdelEcoRI**.

f. pIGHAG1A is digested with XbaI and HindIII. The 1.3 kb fragment containing the anti-HAG gene fused with the C-terminal domain of filamentous phage pIII protein is isolated and ligated with a pre-digested fhag2CdelEcoRI phage vector (XbaI-HindIII; 6.4 kb) to create the vector **fhag1A**

4.2.2: fjun1A (see Figure 12)

a. The EcoRV site of pIG10.3 is converted to a SalI site by oligonucleotide site-directed mutagenesis (Sambrook *et al.*, 1989) with primer:

SalI9-9primer(rev) 5'CTGAATGTCGACATCTTTGTAGTC3'

The mutated pIG10.3 is called **pIG10.3 SalI.**

b. The jun leucine-zipper domain from **pOK1** (Grammatikoff *et al.*, 1994) is amplified by PCR with the primers:

jun2(for):   5'ACGCGTCGACGCCGGTGGTCGGATCGCCCGG3'

jun2(rev):   5'AATTCGGCACCACCGTGGTTCATGACT3'

c. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with SalI and EcoRI, then ligated into pre-digested pIG10.3SalI vector (SalI-EcoRI) to form the vector **jun-pIG10.3SalI.**

d. The vector jun-pIG10.3SalI is digested with XbaI and EcoRI. The 0.14 kb fragment is ligated into the pre-digested vector fhag1A (XbaI-EcoRI; 7kb) to form the vector **fjun1A.**

4.2.3: fjun1B (see Figure 13)

a. The DNA encoding the C-terminal domain including the long linker separating it from the amino terminal domain of the filamentous phage pIII (gIII short) is amplified by PCR using pOK1 (Grammatikoff *et al.*, 1994) as template with the primers:

gIII short(for):   5'GCTTCCGGAGAATTCAATGCTGGCGGCGGCTCT3'

gIII short(rev):   5'CCCCCCCAAGCTTATCAAGACTCCTTATTACG3'

b. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with EcoRI and HindIII, then ligated into pre-digested fhag1A vector (EcoRI-HindIII) to form the vector **fjun1B**.

4.2.4: fpep2_1b, fpep3_1B, fpep10_1b (see Figure 14)

a. These constructs are obtained from a peptide library screened against the intracellular domain of p75, the low affinity receptor of NGF, in a SIP experiment.

b. A peptide library cassette of cyclic peptides with length variants of 6-16 amino acids is prepared from the oligos:

   Groprim: 5'-CATGAATTCGGATCCTCC-3'

   Gron10: 5'-CTATGGCGCGCCTGTCGACTGT(M)$_{6-16}$TGTGGTGGTGGAGGATC-
            CGAATTCATG-3'

where M is a mixture of 19 trinucleotide codons (Virnekäs *et al.*, 1994), excluding the one coding for Cys. The length variation is achieved by coupling 6 trinucleotide positions using the standard coupling procedure, and, for the next 10 coupling cycles, by omitting the capping step during DNA synthesis and by diluting the trinucleotide mixture to achieve stepwise coupling yields of 50%.

The oligos are annealed and filled in with the Klenow fragment of DNA polymerase I to form a double-stranded DNA cassette with standard methods (Sambrook *et al.*, 1989). The cassette is digested with SalI-EcoRI, purified with Qiaex DNA gel extraction kit, and ligated to pre-digested fjun1B vector (SalI-EcoRI) to form the peptide library. The ligated peptide library is transformed into competent DH5a cells harboring pUC18/IMP-p75 (see below) and plated on Luria Broth (LB) (30 µg/ml chloramphenicol + 100 µg/ml ampicillin) and incubated overnight at ambient temperature.

c. The Amp^r Cm^r colonies are scraped with LB, and 1 ml of suspension is used to inoculate 25 ml LB (30 µg/ml chloramphenicol + 100 µg/ml ampicillin + 1 mM IPTG). The culture is incubated overnight at room temperature.

d. The supernatant is separated from the cells by centrifugation (10,000 RPM, 10 min., 4°C). 5 ml of 30% PEG/3M NaCl are added to the supernatant and mixed 100 times. After 1 hour on ice, the phage precipitate is collected by centrifugation (10,000 RPM, 10 min., 4C). The pellet is resuspended in 1 ml TBS buffer. The suspension is filtered with a 0.45 micron filter (Sartorius).

e. 100 µl of log phase K91 cells (or any male E. coli cells (F-pilus containing)) are infected with 10 µl of phage supernatant, plated on LB (30 µg/ml chloramphenicol) and incubated overnight at ambient temperature.

f. Chloramphenicol-resistant transductants are picked, and overnight cultures are prepared to isolate DNA for sequencing. From the sequencing, fpep2_1b, fpep3_1B, fpep10_1b containing peptides pe2, pe3, and pe10 are identified.

pe2: 5'-TGTTTTTTTCGTGGTGGTTTTTTTTAATCATAATCCTCGTTATTGT-3'

   (CysPhePheArgGlyGlyPhePheAsnHisAsnProArgTyrCys)

pe3: 5'-TGTATTGTTTATCATGCTCATTATCTTGTTGCTAAGTGT-3'

   (CysIleValTyrHisAlaHisTyrLeuValAlaLysCys)

pe10: 5'-TGTTCTTATCATCGTCTTTCTACTCGTGTTTGT-3'

   (CysSerTyrHisArgLeuSerThrArgValCys)

4.2.5: fNGF1B (see Figure 15)

a. The DNA encoding the nerve growth factor (NGFI) gene is amplified from pXM NGF (Ibanez et al., 1992) as template with the primers:

NGF(for): 5'AAAAAAGTCGACTCATCCACCCACCCAGTC3'

NGF(rev): 5'AGGAATTCGCCTCTTCTTGCAGCCTT3'

b. The PCR is done following standard protocols (Sambrook et al., 1989). The amplified product is digested with SalI and EcoRI, then ligated into pre-digested fjun1B vector (SalI-EcoRI) to form the vector fNGF1B.

4.2.6: pUC19/IMP-HAG (see Figure 16)

a. The vector f17/9-hag (Krebber et al., 1995) is digested with EcoRI and HindIII. The 1.4 kb fragment containing the gene fusion of the IMP with the HAG peptide, is isolated and cloned into pre-digested **pUC19** (EcoRI-HindIII) to form the vector **pUC19/IMP-HAG**

4.2.7: pUC18/IMP-p75 (see Figure 17)

a. The intracellular domain of p75 containing the C-terminal 142 amino acids is amplified from the cDNA clone of p75 (Chao et al., 1986) as template with the primers:

p75(for): 5' GCTGGCCCGTACGACAAGAGGTGGAACAGCTGC

p75(rev): 5' TCTCGAAGCTTATCACACTGGGGATGTGGC

b. The PCR is done following standard protocols (Sambrook et al., 1989). The amplified product is digested with BsiWI and HindIII, then ligated into pre-digested pUC19 vector (BsiWI-HindIII) to form the vector **pUC19/IMP-p75**.

c. The vector pUC19/IMP-p75 is digested with XbaI and HindIII. The 1 kb fragment is isolated and cloned into the pre-digested **pUC18** vector (XbaI-HindIII) to form the vector **pUC18/IMP-p75**.

4.2.8: pUC18/IMP-IL16 (see Figure 18)

a. The IL16 gene is amplified from the clone **pcDNA3-ILHu1** (M. Baier, Paul Ehrlich Institute, Germany; Baier et al., 1995; Bannert et al., 1996) as template with the primers:

f1Bsu36Ifor: 5'AGACTGCCTCAGGCCAGCCCGACCTCAACTCC3'

f3HindIIIrev2:   5'ATATATAAGCTTTTAGGAGTCTCCAGCAGC3'

b. The PCR is done following standard protocols (Sambrook et al., 1989). The amplified product is digested with Bsu36I and HindIII, then ligated into pre-digested pUC18/IMP-p75 vector (Bsu36I-HindIII) to form the vector **pUC18/IMP-IL16**.

4.3: In vivo SIP with co-transformation and polyphage

4.3.1: Combining 2 libraries (Library 1 is fused with gIII while Library 2 is fused to the IMP).

10 ng each of fjun1B, fjun1A, fpep3_1B, fhag1A, fNGF1B with 500 ng each of pUC18/IMP-p75, pUC18/IMP-HAG, pUC18/IMP-IL16 are co-transformed into DH5a cells by electroporation. The cells are plated on Luria Broth (LB) (30 μg/ml chloramphenicol + 100 μg/ml ampicillin) and incubated overnight at ambient temperature.

The Ampr Cmr colonies are scraped with LB and 1 ml of suspension is used to inoculate 25 ml LB (30 μg/ml chloramphenicol + 100 μg/ml ampicillin + 1 mM IPTG) followed by incubation overnight at room temperature.

4.3.2: In vivo SIP. The supernatant from the cells is separated by centrifugation (10,000 RPM, 10 min., 4°C). 5 ml of 30% PEG/3M NaCl are added to the supernatant and mixed 100 times. After 1 hour on ice, the phage precipitate is collected by centrifugation (10,000 RPM, 10 min., 4°C). The pellet is resuspended in 1 ml TBS buffer, and the suspension is filtered through a 0.45 micron filter (Sartorius).

200 μl of phage supernatant are used to infect 1.8ml of log phase K91 cells (or any male E. coli cells (F-pilus containing)), and the cells are plated on LB (30 μg/ml chloramphenicol + 100 μg/ml ampicillin) and incubated overnight at ambient temperature.

4.3.3: Testing of infectious polyphage DNA patterns and infectity. Twenty individual Ampr Cmr colonies are used to inoculate 5 ml LB (30 μg/ml chloramphenicol + 100 μg/ml ampicillin) in each case and incubated at ambient temperature overnight. Plasmid and RF DNA are isolated from each clone with a Qiagen Miniprep DNA kit. Clones are analysed by restriction analysis with restriction enzymes XbaI and HindIII together with appropriate buffers as supplied and instructed by the manufacturer. The restriction

digests are run in a 0.8% TBE agarose gel at constant voltage of 100V for 1.5 hours. The restriction patterns, together with the relative intensity of the bands (because the phage vectors (fjun1B, fjun1A, fpep3_1B, fNGF1B, fhag1A) have significantly lower copy numbers than the plasmid vectors) allow to identify correctly interacting pairs. For the pair fhag1A+pUC19/IMP-HAG, an XbaI-HindIII digest will yield a 6.5 kb, 3.3 kb, 1.3 kb, and 0.7 kb fragments, while for the pair fpep3_1B+pUC18/IMP-p75, the same digest will yield 6.3 kb, 2.8 kb, 1kb, and 0.7kb fragments. A problem though is to distinguish the potential non-cognate combinations of fjun1B or fjun1A with pUC18/IMP-p75 because they would give similar patterns as the fpep3_1B+pUC18/IMP-p75. To further resolve this, the clones containing identical patterns can be re-digested with BamHI-HindIII. The fjun1A or fjun1B in combination with pUC18/IMP-p75 would yield only 4 fragments - 4.1 kb and 2.9 kb , 2.6 kb , 1.2 kb fragments - while the cognate pair fpep3_1B+pUC18/IMP-p75 will yield 5 fragments - 3.5 kb, 2.9 kb, 2.6 kb, 1.2 kb, 0.5 kb.

To further prove that cognate interacting pairs have been selected, the ability of the clones to form selectively-infective phage particles is tested. Only clones with a cognate pair can form infectious phages. The supernatant from the overnight culture of the individual clones is filtered with a 0.45 micron filter (Sartorius). Ten microliters of phage supernatant are mixed with 100 µl of log phase K91 cells (or any male E. coli cells (F-pilus containing)) for 10 minutes at 37°C. The suspension is plated on LB (30 µg/ml chloramphenicol) and incubated overnight at 37°C. The result is shown in Table 3.b.

In summary (see Figure 19), the results from the above example indicate that among 19 clones analyzed, 8/19 have the cognate pair fpep3_1B+pUC18/IMP-p75 and produce selectively-infective phage; 1/19 has the fhag1A+pUC19/IMP-HAG combination and produces selectively-infective phage.

Example 5: Combination of Multiple Libraries into a Single Phagemid Vector through Recombination, Screening *via* tag system

5.1: Principle (see Figure 20)

To be able to retrieve the genetic information for cognate protein pairs selected *via* a tag fused to one of the partners, two separate libraries in phagemid vectors are constructed containing the *lox* recombination promoting sites and recombined on one phagemid by action of the *cre* recombinase in an *in vivo* recombination.

5.2: Vector construction

Both *lox*P and *lox*P511sites (Hoess *et al.*, 1986) are inserted in tandem into the region flanked by the ColE1 ori and β-lactamase in vector pING1-C1, whereas in vector pONG3-A, the *lox*P site is cloned upstream of the *Xba*I site and the *lox*P511 downstream of the *Hind*III site. Therefore, the genomic DNAs to be cloned are flanked by the *lox*P and *lox*P511 sites.

5.3: Library construction and recombination

The libraries are prepared as in Example 3. The phagemids in the double-resistant clones are recombined through the *cre* recombinase which either is encoded in the phagemid being inducible (Tsurushita *et al.*, 1996), or is transferred through P1 phage infection (Rosner, 1972; Waterhouse *et al.*, 1993). Phages are prepared from the recombined clones by helper phage infection and used to infect new *E. coli* cells (*cre*⁻).

5.4: Selection

The phage particles are prepared from the Cm^R clones and subjected to His-tag selection as in Examples 2 and 3. The sequences encoded in each phagemid, which now contains members of both libraries, can be determined by sequencing using primers specific for myc-tag region (library 1) and His-tag region (library 2).

Example 6: SIP-based library vs. library screening via *in vitro* recombination of separately constructed libraries into one phage vector

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6.1: Principle (see Figure 21)

To be able to retrieve the genetic information for cognate protein pairs selected by SIP interaction *in vivo*, two separate libraries in phage and plasmid vectors are constructed and recombined by co-ligation in an *in vitro* recombination.

6.2: Construction of Libraries A and B

Library A encodes 2 members, namely a single chain Fv antibody against a peptide derived from hemagglutinin (fɑhag) and the leucine zipper domain derived from the jun transcription factor (fjun), both N-terminally fused to the C-terminal domain of gIII from filamentous phage and preceded by the ompA signal sequence followed by the Flag epitope.

Library B encodes 3 members on plasmid vectors of the pUC series, namely the hemagglutinin peptide to which the above ɑhag antibody binds (pUC19-IMPhag), the leucine zipper domain of the fos transcription factor (pUC18-IMPfos) which heterodimerizes with jun via this domain, and the intracellular domain of the low affinity nerve growth factor receptor (pUC18-IMPp75), as a negative control which does not interact with library A members, all fused to the infectivity-mediating N-terminal domains of phage gIII protein, preceded by the gIII signal sequence.

Library A members are cloned into a fd phage vector which also contains downstream of the library A insertion site the N-terminal domains (N1-N2) of gIII, followed by the cloning sites *Bsi*WI and *Hind*III to allow in-frame insertion of library B members.

Library A construct fɑhag is identical to the f17/9-hag fd phage vector (Krebber *et al.*, 1995) and serves as basis for construction of fjun. The jun leucine zipper together with amino acids 290 to 326 of the C-terminal part of gIII is PCR-amplified (primers FR620 and FR621, containing *Eco*RV and *Sfi*I sites, respectively) from the construct fjun1B (containing the jun leucine zipper fused to amino acids 290 to 493 of gIII) generated in Example 4. The resulting PCR fragment is ligated directionally into EcoRV/SfiI-digested

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f17/9-hag vector in frame with amino acids 327 to 493 of the gIII C-terminal domain resulting in vector **fjunhag** (see Figure 22).

Generation of library B constructs pUC19-IMPhag and pUC18-IMPp75 is described in Example 4. To construct pUC18-IMPfos, amino acids 219 to 272 of the N-terminal part of gIII together with the fos leucine zipper are PCR-amplified (primers FR618 and FR619, containing BsiWI and HindIII sites, respectively) from the pOK1 phagemid vector (Grammatikoff et al., 1994). The resulting PCR fragment is ligated directionally into BsiWI/HindIII-digested pUC18-IMPp75 to create **pUC18-IMPfos** (see Figure 17).

Primers:

FR618: 5'CGCCGTACGGCGGCTCTGGTGGTGGTTCTGGTGGC3'

FR619: 5'CCCAAGCTTTTAGACTAGCTGACTAGAAGATCTGC3'

FR620: 5'CGCGATATCGTCGACGCCGGTGGTCGGATCGCC3'

FR621: 5'CGCGGCCCCCGAGGCCCCACCACCGGAACCGCCTCCC3'

6.3: Preparation and recombination of library A and B and selection of interacting protein pairs by SIP

Non-covalent, cognate interactions of αhag antibody with hag peptide (Krebber et al. 1995) and of fos and jun leucine zipper domains (Grammatikoff et al., 1994) generates infective SIP phage. Thus, from the six possible combinations of members of the model libraries A and B (**fαhag-hag**, fαhag-fos, fαhag-p75, **fjun-fos**, fjun-hag, fjun-p75), only two combinations (cognate pairs in bold) should be selected by in vivo SIP. To recombine the library members in all possible permutations, library A is linearized by digestion with BsiWI/HindIII to prepare it for random incorporation of library B members, prepared by mass-excision with BsiWI/HindIII from the construct B pool described above. After co-ligation of the mass-excised library B fragments into library A vectors, the sample is transformed into competent E.coli cells, plated onto chloramphenicol-containing LB agar plates and grown overnight at 37°C. The recombined library size can be determined by plating serial dilutions of the transformation and can be compared to

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the complexities of the individual libraries A and B. The total recombined library is scraped from the plates in LB medium and used to inoculate an appropriate volume of chloramphenicol-selective LB-medium supplemented with 1 mM IPTG. After growth at 30°C overnight with constant shaking to allow production of SIP phages, the bacteria are pelleted by centrifugation and phages present in the supernatant are precipitated on ice for one hour by addition of 0.25 volumes of 20% PEG/2.5 M NaCl. The phages are pelleted by centrifugation for 30 min at 10 000 x g and 4°C. The pellet is resuspended in an appropriate volume of 1 x TBS buffer and filtered through a 0.45 µM filter. Serial dilutions of this filtrate are used to infect F^+ E.coli cells. The double-stranded, replicative form phage DNA is prepared from resulting transductant colonies by standard methods and analyzed by restriction digest and sequencing for the presence and identity of library A and B members. Furthermore, the supernatant of transductant colonies is analyzed for the presence of infective SIP phages to confirm that protein-protein interaction of a particular pair selected from the recombined libraries A and B is responsible for SIP phage infectivity.

Alternatively, the model libraries A (2 members) and B (3 members) are used to construct all possible combinations (listed above) individually, and equal amounts (50 ng) of each of the 6 combinations can be co-transformed into competent E. coli cells followed by the steps listed above. The distribution of individual constructs after co-transformation as well as the distribution of transductants resulting from the model library can be analyzed as described above. The selective recovery of phage constructs which co-encode cognate protein pairs demonstrates the feasibility of SIP-based selection of binding partners after an appropriate recombination event.

Example 7: 'Spatial' *in vivo* SIP

7.1: Principle (see Figure 23)

Coupling of information about members of interacting peptides or proteins is achieved by having a spatial relationship between the particles displaying the selectable or

screenable property (in this example phages for the SIP experiment) and the package containing the genetic information for the individual library members (in this example the *E. coli* cell secreting the phage particle being screened), i. e. a correlation between the phage being examined and the position of the corresponding *E. coli* host on the master plate.

7.2: Combining 2 libraries (Library A is fused with gIII while library B is fused to the IMP)

10 ng each of fjun1B, fjun1A, fpep3_1B, fhag1A, fNGF1B are co-transformed with 500 ng each of pUC18/IMP-p75, pUC19/IMP-HAG, pUC18/IMP-IL16 into DH5a cells by electroporation. The transformants are plated on LB (30 µg/ml chloramphenicol + 100 µg/ml ampicillin) and incubated overnight at ambient temperature.

7.3: Screening of co-transformants by SIP

From the master plate of co-transformants, each of the co-transformants are labelled and inoculated separately into 5 ml LB (30 µg/ml chloramphenicol + 100 µg/ml ampicillin) and incubated overnight at ambient temperature.

Plasmid and RF DNA are isolated from each clones with a Qiagen Miniprep DNA kit. Clones are analysed by restriction analysis with restriction enzymes XbaI and HindIII together with appropriate buffers as supplied and instructed by the manufacturer. The restriction digests are run in a 0.8% TBE agarose gel at constant voltage of 100 V for 1 to 2 hours. Restriction patterns allow discrimination of the particular clones.

The supernatant from the overnight culture of the individual clones is filtered with a 0.45 micron filter (Sartorius). Ten microliters of phage supernatant are mixed with 100 µl of log phase K91 cells (or any male E. coli cells (F-pilus containing)) for 10 minutes at 37°C. The suspension is plated on LB (30ug/ml chloramphenicol) and incubated overnight at 37°C.

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A positive co-transformant (i.e. contains the correct interacting pair) has a corresponding correct restriction pattern and is capable of producing infectious phages, that are incapable of secondary or subsequent infections. Polyphage particles being capable of such infections, and containing the genetic information of an interacting pair as well, can readily be identified by their restriction digest pattern.

Example 8: *E. coli* display

8.1: Principle (see Figure 24)

Two libraries are introduced into *E.coli* cells, with expressed members of library A (such as antibody, peptide, or cDNA libraries) being presented at the surface of the cells. In those cases where interacting pairs are formed, members of library B (such as antibody, peptide, or cDNA libraries) are transported in the complex with its cognate partner to the surface of the cell as well, thus displaying a selectable or screenable property such as a tag. Selected cells contain the information for both interacting partners.

8.2: Preparation of Library A

A thioredoxin peptide library is prepared as fusions to the *E. coli* flagellin in the pFLITRX vector essentially as described (Lu *et al.*, 1995).

8.3: Preparation of Library B

An cyclic, variable-length peptide library including a FLAG epitope (Hopp *et al.*, 1988; Knappik and Plückthun, 1994) is prepared essentially as described in Example 4.2.4, and cloned in the pTERM vector, a modified version of the pto2H10a3s vector (Krebber *et al.*, 1996) containing a chloramphenicol-resistance gene instead of the kanamycin-resistance gene. The pTERM vector can be assembled by standard methods starting from pto2H10a3s. This cyclic peptide library is packaged by infection with a helper phage (M13K07 or VCSM13) by standard methods (Sambrook *et al.*, 1989).

8.4: Combination of Library A and Library B

An aliquot of the *E. coli* cells containing Library A is used to inoculate 50 ml LB (100 µg/ml ampicillin) and incubated at ambient temperature until the OD600 reached 0.4. The cells are infected with phages containing Library B at a multiplicity of infection (MOI) of 10. After 30 min of infection, the cells are collected by centrifugation (5000 RPM, 10 minutes, 4°C) and resuspended in 1 ml LB. The suspension is plated on M9 media (+ 1 mM MgCl2, supplemented with 0.5% glucose, 0.2% casamino acids, 100 µg/ml ampicillin, 30 µg/ml chloramphenicol).

8.5: Selection of interacting pairs

The Ampr Cmr colonies are scraped with M9 media (+ 1 mM MgCl2, supplemented with 0.5% glucose, 0.2% casamino acids, 100 µg/ml ampicillin, 30 µg/ml chloramphenicol), and an aliquot of the suspension is used to inoculate 25 ml M9 media (+ 1 mM MgCl2, supplemented with 0.5% glucose, 0.2% casamino acids, 100 µg/ml ampicillin, 30 µg/ml chloramphenicol) and incubated at 37°C until saturation. Selection is performed essentially as described (Lu *et al.*, 1995), the modification being that the antibody used for selection is the M1 anti-FLAG antibody (Kodak).

Individual enriched Ampr Cmr colonies are isolated and the sequences of the corresponding interacting peptide(s) and cyclic peptide(s) are determined by DNA sequencing. To confirm that the encoded peptide and cyclic peptide form a cognate pair, each of the clones is tested for enrichment based on the selection method described above, whereby the Ampr Cmr colonies bind to the M1 anti-FLAG antibody in a single round of selection.

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CLAIMS

1. A method for identifying a plurality of nucleic acid sequences, said nucleic acid sequences each encoding a (poly)peptide capable of interacting with at least one further (poly)peptide encoded by a different member of said plurality of nucleic acid sequences, comprising the steps of:

    (a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides;

    (b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with further (poly)peptides as mentioned in step (a), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in step (a) and wherein at least one of said properties displayed by each of said vector molecules and/or the recombinant inserts used in steps (a) and (b), upon the interaction of a (poly)peptide from said first library with a (poly)peptide from said second library together generate a screenable or selectable property;

    (c) optionally, providing additional libraries of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with or causing interaction of (a) further (poly)peptide(s) as mentioned in step (a) and/or step (b), wherein the vector molecules employed for the

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production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in steps (a) and (b) and, optionally, at least one of said properties displayed by said vector molecule and/or the recombinant inserts used in step (c) together with at least one of said properties displayed by either said vector molecule and/or said recombinant inserts used in steps (a) and/or (b), upon the interaction of a (poly)peptide from said additional library with either a (poly)peptide from said first library and/or a (poly)peptide from said second library generate a screenable or selectable property;

(d) expressing members of said libraries of recombinant vectors or nucleic acid sequences mentioned in steps (a), (b) and optionally (c), in appropriate host cells so that at least one interaction is established;

(e) selecting for the generation of said screenable or selectable property representing the interaction of said (poly)peptides;

(f) optionally, carrying out further screening, selection and/or purification steps; and

(g) identifying said nucleic acid sequences encoding said (poly)peptides.

2.   A method for identifying a plurality of nucleic acid sequences, said nucleic acid sequences each encoding a (poly)peptide capable of interacting with at least one further (poly)peptide encoded by a different member of said plurality of nucleic acid sequences, comprising the steps of:

(a)   expressing in appropriate host cells

(aa)   nucleic acid sequences contained in a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides;

(ab)   nucleic acid sequences contained in a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with further (poly)peptides as mentioned in step (aa), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in step (aa) and wherein at least one of said properties displayed by each of said vector molecules and/or the recombinant inserts used in steps (aa) and (ab), upon the interaction of a (poly)peptide from said first library with a (poly)peptide from said second library together generate a screenable or selectable property;

(ac)   optionally, nucleic acid sequences contained in additional libraries of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences encoding (poly)peptides capable of interacting with or causing interaction of (a) further (poly)peptide(s) as mentioned in step (aa) and/or step (ab), wherein the vector molecules employed for the production of said recombinant vector molecules and/or the recombinant inserts display properties that are phenotypically distinguishable from those of the vector molecules and/or the recombinant inserts used in steps (aa) and (ab) and, optionally, at least one of said properties displayed by said vector molecule and/or the recombinant inserts used in step (ac) together with at least one of said properties displayed by either said vector molecule and/or said recombinant inserts used in steps (aa) and/or (ab), upon the

interaction of a (poly)peptide from said additional library with either a (poly)peptide from said first library and/or a (poly)peptide from said second library generate a screenable or selectable property;

so that at least one interaction is established;

(b) selecting for the generation of said screenable or selectable property representing the interaction of said (poly)peptides;

(c) optionally, carrying out further screening, selection and/or purification steps; and

(d) identifying said nucleic acid sequences encoding said (poly)peptides.

3. The method according to claim 1 or 2, wherein said screenable or selectable property is expressed extracellularly.

4. The method according to any one of claims 1 to 3 wherein said recombinant vector molecules in step (a)/(aa) give rise to a replicable genetic package (RGP) displaying said (poly)peptides at its surface.

5. The method according to claim 4, wherein said recombinant vector molecule is a recombinant phage, phagemid or virus.

6. The method according to claim 5, wherein said phage is
    (a) one of the class I phage fd, M13, If, Ike, ZJ/2, Ff;
    (b) one of the class II phage Xf, Pf1, and Pf3;
    (c) one of the lambdoid phages, lambda, 434, P1;
    (d) one of the class of enveloped phages, PRD1; or
    (e) one of the class paramyxo-viruses, orthomyxo-viruses, baculo-viruses, retro-viruses, reo-viruses and alpha-viruses.

7. The method according to any one of claims 4 to 6, wherein said selection step (e)/(b) is carried out by selecting polyphage comprising the interacting (poly)peptides.

8. The method according to any one of claims 4 to 7, wherein said screenable or selectable property is connected to the infectivity of said RGP.

9. The method according to claim 8, wherein said RGP is encoded by said recombinant vector used in step (a)/(aa) and rendered non-infective and infectivity of said RGP is restored by interaction of said (poly)peptide of step (a)/(aa) with the (poly)peptide of step (b)/(ab) and/or (c)/(ac), said (poly)peptide of step (b)/(ab) and/or (c)/(ac) being fused to a domain that confers infectivity to said RGP.

10. The method according to claim 9, wherein said RGP is rendered non-infective by modification of a genetic sequence which encodes a surface protein necessary for the RGP's binding to and infection of a host cell.

11. The method according to any one of claims 1 to 3, wherein said recombinant vector molecules in step (a)/(aa) give rise to a fusion protein which is expressed on the surface of a cell, preferably a bacterium.

12. The method according to claim 11, wherein said bacterium is Neisseria gonorrhoe or E. coli and said fusion protein consists of at least a part of a flagellum, lam B, peptidoglycan-associated lipoprotein or the Omp A protein and said (poly)peptide.

13.   The method according to any one of claims 3 to 7, 11 or 12, wherein said (poly)peptides encoded by said recombinant vector molecules of step (b)/(ab) or (c)/(ac) are linked to at least one screenable or selectable tag.

14.   The method according to claim 13, wherein said screenable or selectable tag is encoded by said recombinant vector of step (b)/(ab) or (c)/(ac).

15.   The method according to claim 13 or 14, wherein said screenable or selectable tag is selected from the list His(n), myc, FLAG, malE, thioredoxin, GST, streptavidin, beta-galactosidase, alkaline phosphatase, T7 gene 10, Strep-tag and calmodulin.

16.   The method according to claim 13, wherein said screenable or selectable tag is encoded by the genome of the host cell.

17.   The method according to any one of claims 1 to 16, wherein said (poly)peptides encoded by the nucleic acid sequences of said additional libraries of step (c)/(ac) cause the interaction of said (poly)peptides of steps (a)/(aa) and (b)/(ab) via phosphorylation, glycosylation, methylation, lipidation or farnesylation of at least one of said (poly)peptides of steps (a)/(aa) and (b)/(ab).

18.   The method according to any of claims 1 to 10 and 13 to 17, wherein said host cells in step (d)/(a) are spatially addressable, and the nucleic acid sequences mentioned in step (g)/(d) are retrieved from the corresponding spatially addressable host cell.

19.   The method according to claim 1 or 2, wherein said screenable or selectable property is expressed intracellularly.

20. The method according to claim 19, wherein said screenable or selectable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

21. The method according to any one of claims 1 to 20, wherein said recombinant vectors of step (a)/(aa), (b)/(ab) and (c)/(ac) comprise recombination promoting sites and in said step (e)/(b) recombination events are selected for, wherein said nucleic acid sequences encoding said (poly)peptides of step (a)/(aa), said nucleic acid sequences encoding said (poly)peptides of step (b)/(ab) and optionally said nucleic acid sequences encoding said (poly)peptides of step (c)/(ac) are contained in the same vector.

22. The method according to claim 21, wherein said recombination events are mediated by the site-specific recombination mechanisms Cre-lox, attP-attB, Mu gin or yeast flp.

23. The method according to claim 21 wherein said recombination promotion sites are restriction enzyme recognition sites and said recombination event is achieved by cutting the recombinant vector molecules mentioned in step (a)/(aa), (b)/(ab) and optionally (c)/(ac) with at least two different restriction enzymes and effecting recombination of the nucleic acid sequences contained in said vectors by ligation.

24. The method according to any one of claims 1 to 23 wherein said identification of said nucleic acid sequences is effected after the selection of step (e)/(b) via PCR and preferably sequencing of said nucleic acid sequences after said PCR.

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25. The method according to any one of claims 1 to 24, wherein said recombinant vectors of step (a)/(aa), (b)/(ab) and/or (c)/(ac) comprise at least one gene encoding a selection marker.

26. The method according to claim 25, wherein said selection marker is a resistance to an antibiotic, preferably to ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

27. The method according to any one of claims 1 to 26, wherein said host cells are F' and preferably E.coli XL-1 Blue, K91 or its derivatives, TG1, XL1kan or TOP10F.

28. The method according to any one of claims 3 to 18 and 21 to 27, wherein said RGPs are produced with the use of helper phage taken from the list R408, M13k07 and VCSM13, M13de13, fCA55 and fKN16 or derivatives thereof.

29. The method according to any of claims 1 to 28, wherein at least one of said genetically diverse nucleic acid sequences encode members of the immunoglobulin superfamily.

30. The method according to claim 29, wherein said genetically diverse nucleic acid sequences encode a repertoire of immunoglobulin heavy or light chains.

31. The method according to any of claims 1 to 30, in which said genetically diverse nucleic acid sequences are generated by a mutagenesis method.

32. The method according to any of claims 1 to 31, in which said genetically diverse nucleic acid sequences are generated from a cDNA library.

33.   The method according to any one of claims 1 to 32 wherein said nucleic acid sequences are genes or parts thereof.

34.   Kit comprising at least

(a)   a recombinant vector molecule as described in step (a)/(aa) or a corresponding vector molecule;

(b)   a recombinant vector molecule as described in step (b)/(ab) or a corresponding vector molecule; and, optionally,

(c)   at least one further recombinant vector molecule as described in step (c)/(ac) or a corresponding vector molecule.

Figure 1: General description of the polyphage principle
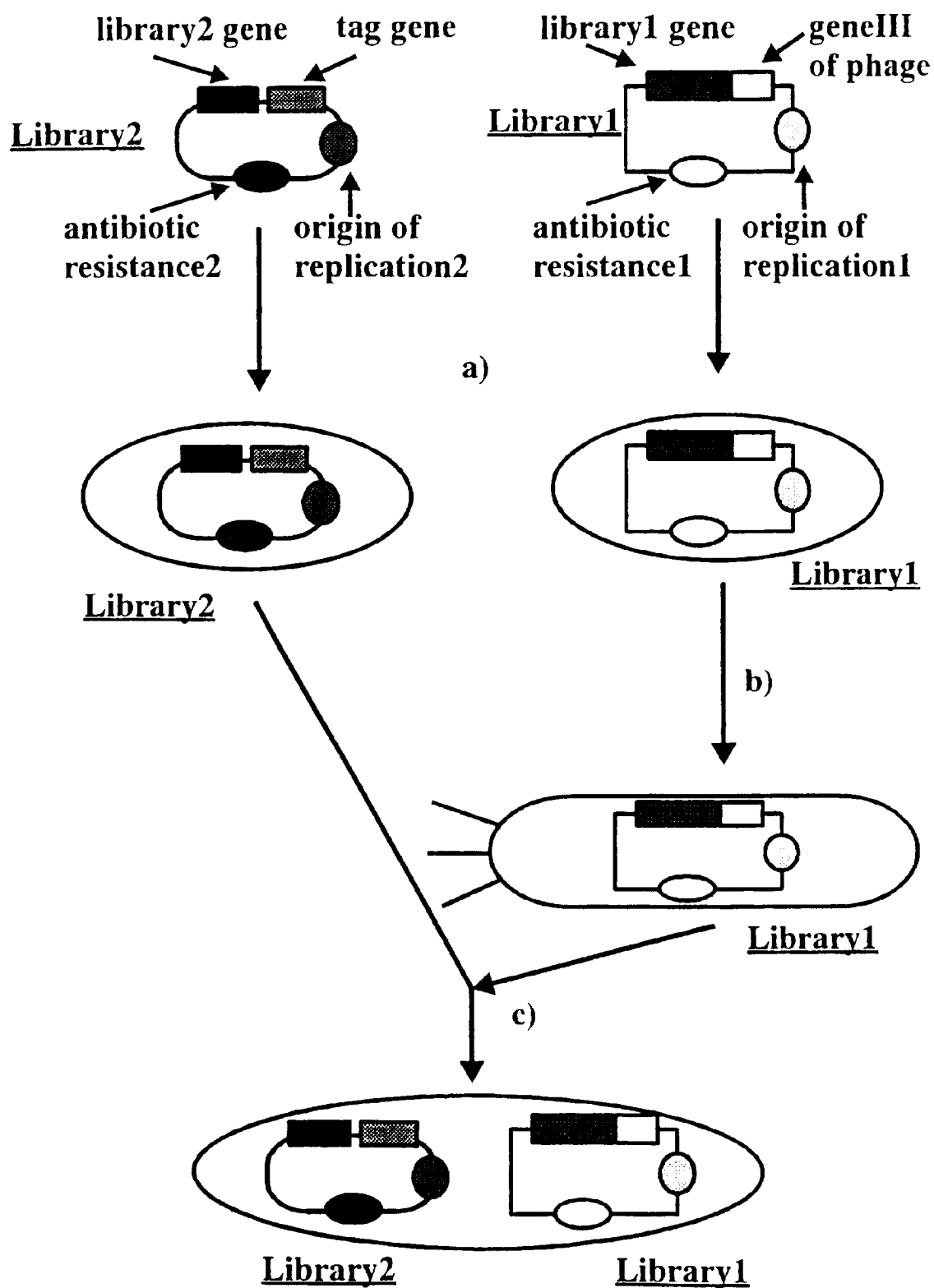
Figure 1: General description of the polyphage principle (cont.)
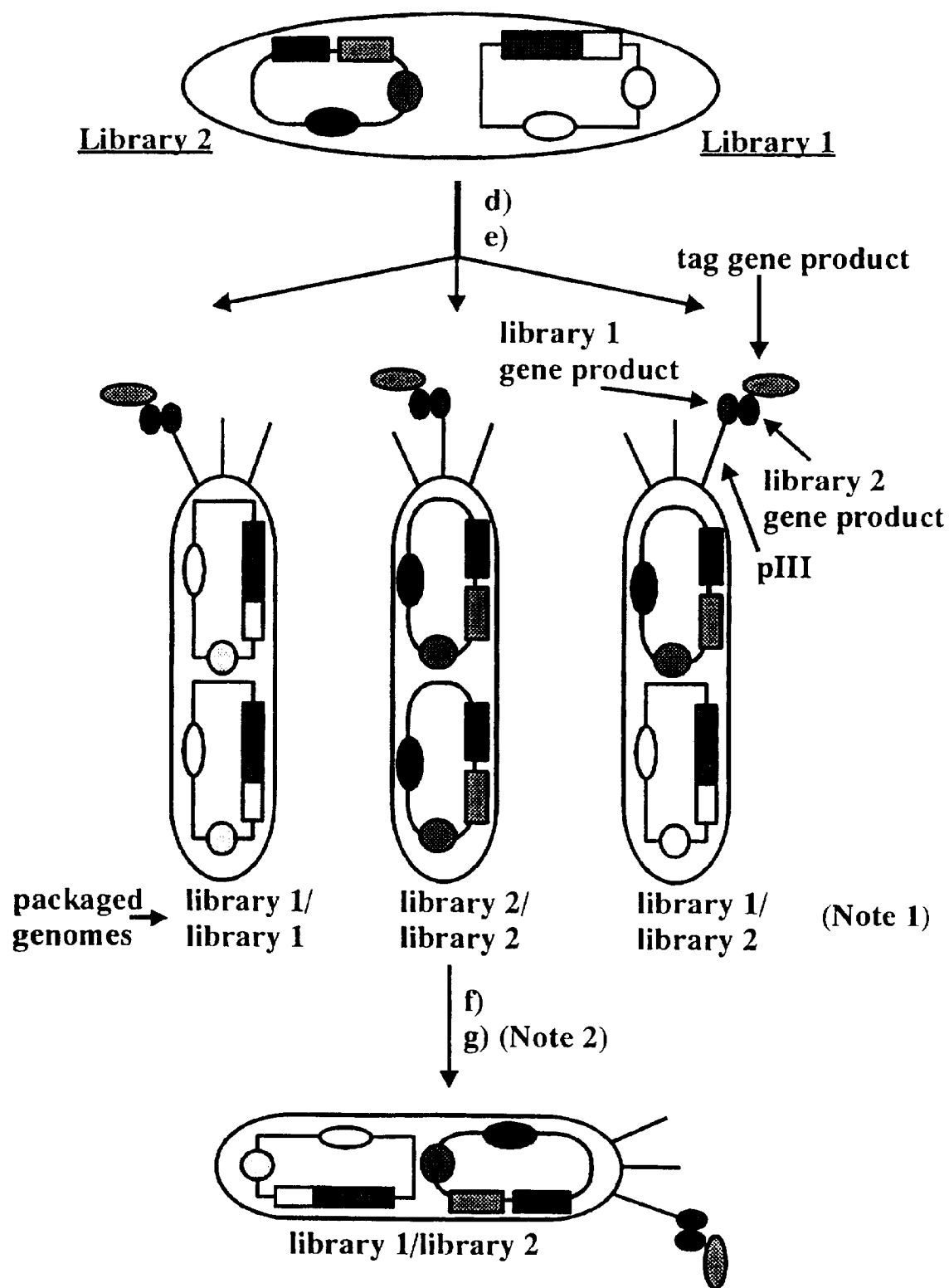
Figure 2: Co-transformation of two phagemids, polyphage formation and selection *via* His-tag: general description

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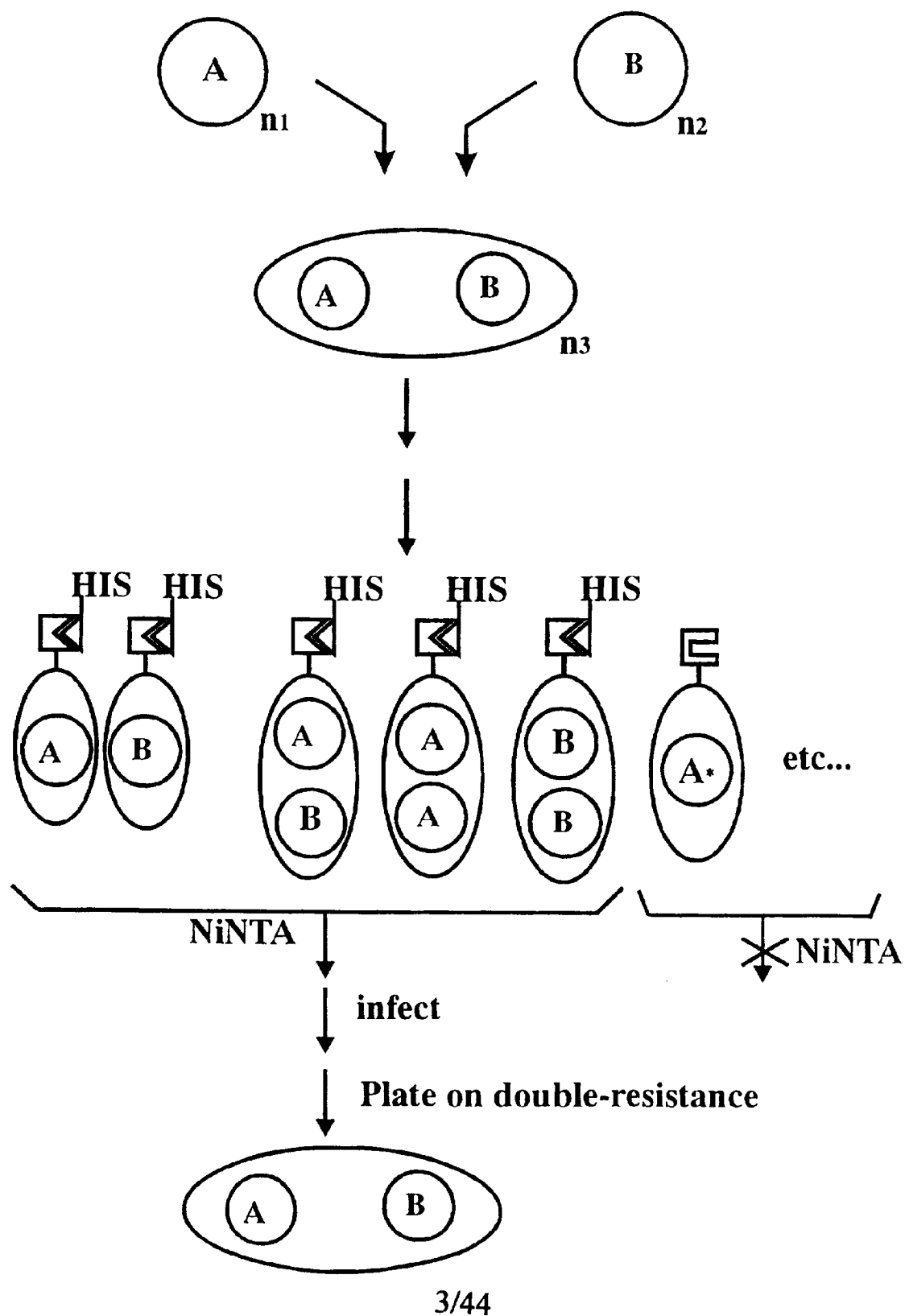
Figure 3: pBS vector series: functional map and sequence of pBS13
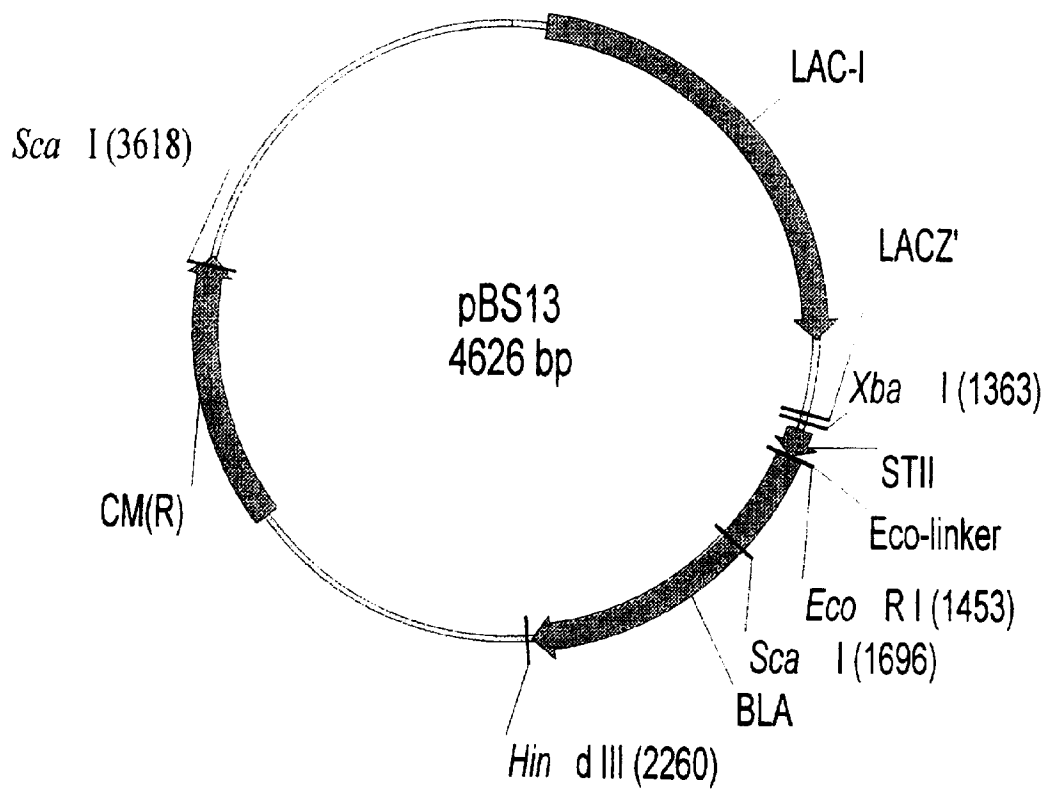
Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
  1   ACCCGACACC ATCGAATGGC GCAAAACCTT TCGCGGTATG GCATGATAGC
      TGGGCTGTGG TAGCTTACCG CGTTTTGGAA AGCGCCATAC CGTACTATCG

 51   GCCCGGAAGA GAGTCAATTC AGGGTGGTGA ATGTGAAACC AGTAACGTTA
      CGGGCCTTCT CTCAGTTAAG TCCCACCACT TACACTTTGG TCATTGCAAT

101   TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG TTTCCCGCGT
      ATGCTACAGC GTCTCATACG GCCACAGAGA ATAGTCTGGC AAAGGGCGCA

151   GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG
      CCACTTGGTC CGGTCGGTGC AAAGACGCTT TTGCGCCCTT TTTCACCTTC

201   CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACTG
      GCCGCTACCG CCTCGACTTA ATGTAAGGGT TGGCGCACCG TGTTGTTGAC

251   GCGGGCAAAC AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT
      CGCCCGTTTG TCAGCAACGA CTAACCGCAA CGGTGGAGGT CAGACCGGGA

301   GCACGCGCCG TCGCAAATTG TCGCGGCGAT TAAATCTCGC GCCGATCAAC
      CGTGCGCGGC AGCGTTTAAC AGCGCCGCTA ATTTAGAGCG CGGCTAGTTG

351   TGGGTGCCAG CGTGGTGGTG TCGATGGTAG AACGAAGCGG CGTCGAAGCC
      ACCCACGGTC GCACCACCAC AGCTACCATC TTGCTTCGCC GCAGCTTCGG

401   TGTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA GTGGGCTGAT
      ACATTTCGCC GCCACGTGTT AGAAGAGCGC GTTGCGCAGT CACCCGACTA

451   CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
      GTAATTGATA GGCGACCTAC TGGTCCTACG GTAACGACAC CTTCGACGGA

501   GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC
      CGTGATTACA AGGCCGCAAT AAAGAACTAC AGAGACTGGT CTGTGGGTAG

551   AACAGTATTA TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA
      TTGTCATAAT AAAAGAGGGT ACTTCTGCCA TGCGCTGACC CGCACCTCGT

601   TCTGGTCGCA TTGGGTCACC AGCAAATCGC GCTGTTAGCG GGCCCATTAA
      AGACCAGCGT AACCCAGTGG TCGTTTAGCG CGACAATCGC CCGGGTAATT

651   GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG GCTGGCATAA ATATCTCACT
      CAAGACAGAG CCGCGCAGAC GCAGACCGAC CGACCGTATT TATAGAGTGA

701   CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT GGAGTGCCAT
      GCGTTAGTTT AAGTCGGCTA TCGCCTTGCC CTTCCGCTGA CCTCACGGTA

751   GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA
      CAGGCCAAAA GTTGTTTGGT ACGTTTACGA CTTACTCCCG TAGCAAGGGT

801   CTGCGATGCT GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC
      GACGCTACGA CCAACGGTTG CTAGTCTACC GCGACCCGCG TTACGCGCGG
```

Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
 851   ATTACCGAGT CCGGGCTGCG CGTTGGTGCG GACATCTCGG TAGTGGGATA
       TAATGGCTCA GGCCCGACGC GCAACCACGC CTGTAGAGCC ATCACCCTAT

 901   CGACGATACC GAAGACAGCT CATGTTATAT CCCGCCGTTA ACCACCATCA
       GCTGCTATGG CTTCTGTCGA GTACAATATA GGGCGGCAAT TGGTGGTAGT

 951   AACAGGATTT TCGCCTGCTG GGGCAAACCA GCGTGGACCG CTTGCTGCAA
       TTGTCCTAAA AGCGGACGAC CCCGTTTGGT CGCACCTGGC GAACGACGTT

1001   CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC CCGTCTCACT
       GAGAGAGTCC CGGTCCGCCA CTTCCCGTTA GTCGACAACG GGCAGAGTGA

1051   GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC
       CCACTTTTCT TTTTGGTGGG ACCGCGGGTT ATGCGTTTGG CGGAGAGGGG

1101   GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG
       CGCGCAACCG GCTAAGTAAT TACGTCGACC GTGCTGTCCA AAGGGCTGAC

1151   GAAAGCGGGC AGTGAGCGGT ACCCGATAAA AGCGGCTTCC TGACAGGAGG
       CTTTCGCCCG TCACTCGCCA TGGGCTATTT TCGCCGAAGG ACTGTCCTCC

1201   CCGTTTTGTT TTGCAGCCCA CCTCAACGCA ATTAATGTGA GTTAGCTCAC
       GGCAAAACAA AACGTCGGGT GGAGTTGCGT TAATTACACT CAATCGAGTG

1251   TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT
       AGTAATCCGT GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA

1301   GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT
       CACCTTAACA CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GATACTGGTA

                  XbaI
                  ~~~~~~
1351   GATTACGAAT TCTAGAGGT TGAGGTGATT TTATGAAAAA GAATATCGCA
       CTAATGCTTA AAGATCTCCA ACTCCACTAA AATACTTTTT CTTATAGCGT

1401   TTTCTTCTTG CATCTATGTT CGTTTTTTCT ATTGCTACAA ATGCATACGC
       AAAGAAGAAC GTAGATACAA GCAAAAAAGA TAACGATGTT TACGTATGCG

       EcoRI
       ~~~~~~
1451   TGAATTCCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT
       ACTTAAGGTG GGTCTTTGCG ACCACTTTCA TTTTCTACGA CTTCTAGTCA

1501   TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC
       ACCCACGTGC TCACCCAATG TAGCTTGACC TAGAGTTGTC GCCATTCTAG

1551   CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA
       GAACTCTCAA AAGCGGGGCT TCTTGCAAAA GGTTACTACT CGTGAAAATT

1601   AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC
       TCAAGACGAT ACACCGCGCC ATAATAGGGC ATAACTGCGG CCCGTTCTCG
```

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Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
                                                               ScaI
                                                             ~~~~~~
    1651  AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA
          TTGAGCCAGC GGCGTATGTG ATAAGAGTCT TACTGAACCA ACTCATGAGT

    1701  CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG
          GGTCAGTGTC TTTTCGTAGA ATGCCTACCG TACTGTCATT CTCTTAATAC

    1751  CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA
          GTCACGACGG TATTGGTACT CACTATTGTG ACGCCGGTTG AATGAAGACT

    1801  CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG
          GTTGCTAGCC TCCTGGCTTC CTCGATTGGC GAAAAAACGT GTTGTACCCC

    1851  GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT
          CTAGTACATT GAGCGGAACT AGCAACCCTT GGCCTCGACT TACTTCGGTA

    1901  ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT
          TGGTTTGCTG CTCGCACTGT GGTGCTACGG ACATCGTTAC CGTTGTTGCA

    1951  TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA
          ACGCGTTTGA TAATTGACCG CTTGATGAAT GAGATCGAAG GGCCGTTGTT

    2001  TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC
          AATTATCTGA CCTACCTCCG CCTATTTCAA CGTCCTGGTG AAGACGCGAG

    2051  GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC
          CCGGGAAGGC CGACCGACCA AATAACGACT ATTTAGACCT CGGCCACTCG

    2101  GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC
          CACCCAGAGC GCCATAGTAA CGTCGTGACC CCGGTCTACC ATTCGGGAGG

    2151  CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG
          GCATAGCATC AATAGATGTG CTGCCCCTCA GTCCGTTGAT ACCTACTTGC

    2201  AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAT
          TTTATCTGTC TAGCGACTCT ATCCACGGAG TGACTAATTC GTAACCATTA

                HindIII
                ~~~~~~~
    2251  GAGCATGCAA GCTTGACCTG TGAAGTGAAA AATGGCGCAC ATTGTGCGAC
          CTCGTACGTT CGAACTGGAC ACTTCACTTT TTACCGCGTG TAACACGCTG

    2301  ATTTTTTTTG TCTGCCGTTT ACCGCTACTG CGTCACGGAT CCCCACGCGC
          TAAAAAAAAC AGACGGCAAA TGGCGATGAC GCAGTGCCTA GGGGTGCGCG

    2351  CCTGTAGCGG CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG
          GGACATCGCC GCGTAATTCG CGCCGCCCAC ACCACCAATG CGCGTCGCAC

    2401  ACCGCTACAC TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC
          TGGCGATGTG AACGGTCGCG GGATCGCGGG CGAGGAAAGC GAAAGAAGGG
```

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Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
2451   TTCCTTTCTC GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG
       AAGGAAAGAG CGGTGCAAGC GGCCGAAAGG GGCAGTTCGA GATTTAGCCC

2501   GCATCCCTTT AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA
       CGTAGGGAAA TCCCAAGGCT AAATCACGAA ATGCCGTGGA GCTGGGGTTT

2551   AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC
       TTTGAACTAA TCCCACTACC AAGTGCATCA CCCGGTAGCG GGACTATCTG

2601   GGTTTTTCGC CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT
       CCAAAAAGCG GGAAACTGCA ACCTCAGGTG CAAGAAATTA TCACCTGAGA

2651   TGTTCCAAAC TGGAACAACA CTCAACCCTA TCTCGGTCTA TTCTTTTGAT
       ACAAGGTTTG ACCTTGTTGT GAGTTGGGAT AGAGCCAGAT AAGAAAACTA

2701   TTATAAGGGA TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT
       AATATTCCCT AAAACGGCTA AAGCCGGATA ACCAATTTTT TACTCGACTA

2751   TTAACAAAAA TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT
       AATTGTTTTT AAATTGCGCT TAAAATTGTT TTATAATTGC AAATGTTAAA

2801   CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT
       GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA

2851   TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGTCGAGAC GTTGGGTGAG
       AAGATTTATG TAAGTTTATA CATAGGCGAG TACAGCTCTG CAACCCACTC

2901   GTTCCAACTT TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT
       CAAGGTTGAA AGTGGTATTA CTTTATTCTA GTGATGGCCC GCATAAAAAA

2951   GAGTTATCGA GATTTTCAGG AGCTAAGGAA GCTAAATGG AGAAAAAAAT
       CTCAATAGCT CTAAAAGTCC TCGATTCCTT CGATTTACC TCTTTTTTA

3001   CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT AAAGAACATT
       GTGACCTATA TGGTGGCAAC TATATAGGGT TACCGTAGCA TTTCTTGTAA

3051   TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG
       AACTCCGTAA AGTCAGTCAA CGAGTTACAT GGATATTGGT CTGGCAAGTC

3101   CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT
       GACCTATAAT GCCGGAAAAA TTTCTGGCAT TTCTTTTTAT TCGTGTTCAA

3151   TTATCCGGCC TTTATTCACA TTCTTGCCCG CCTGATGAAT GCTCATCCGG
       AATAGGCCGG AAATAAGTGT AAGAACGGGC GGACTACTTA CGAGTAGGCC

3201   AGTTCCGTAT GGCAATGAAA GACGGTGAGC TGGTGATATG GGATAGTGTT
       TCAAGGCATA CCGTTACTTT CTGCCACTCG ACCACTATAC CCTATCACAA

3251   CACCCTTGTT ACACCGTTTT CCATGAGCAA ACTGAAACGT TTTCATCGCT
       GTGGGAACAA TGTGGCAAAA GGTACTCGTT TGACTTTGCA AAAGTAGCGA
```

Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
3301   CTGGAGTGAA  TACCACGACG  ATTTCCGGCA  GTTTCTACAC  ATATATTCGC
       GACCTCACTT  ATGGTGCTGC  TAAAGGCCGT  CAAAGATGTG  TATATAAGCG

3351   AAGATGTGGC  GTGTTACGGT  GAAAACCTGG  CCTATTTCCC  TAAAGGGTTT
       TTCTACACCG  CACAATGCCA  CTTTTGGACC  GGATAAAGGG  ATTTCCCAAA

3401   ATTGAGAATA  TGTTTTTCGT  CTCAGCCAAT  CCCTGGGTGA  GTTTCACCAG
       TAACTCTTAT  ACAAAAAGCA  GAGTCGGTTA  GGGACCCACT  CAAAGTGGTC

3451   TTTTGATTTA  AACGTGGCCA  ATATGGACAA  CTTCTTCGCC  CCCGTTTTCA
       AAAACTAAAT  TTGCACCGGT  TATACCTGTT  GAAGAAGCGG  GGGCAAAAGT

3501   CCATGGGCAA  ATATTATACG  CAAGGCGACA  AGGTGCTGAT  GCCGCTGGCG
       GGTACCCGTT  TATAATATGC  GTTCCGCTGT  TCCACGACTA  CGGCGACCGC

3551   ATTCAGGTTC  ATCATGCCGT  CTGTGATGGC  TTCCATGTCG  GCAGAATGCT
       TAAGTCCAAG  TAGTACGGCA  GACACTACCG  AAGGTACAGC  CGTCTTACGA

                   ScaI
                   ~~~~~~
3601   TAATGAATTA  CAACAGTACT  GCGATGAGTG  GCAGGGCGGG  GCGTAATTTT
       ATTACTTAAT  GTTGTCATGA  CGCTACTCAC  CGTCCCGCCC  CGCATTAAAA

3651   TTTAAGGCAG  TTATTGGTGC  CCTTAAACGC  CTGGTGCTAC  GCCTGAATAA
       AAATTCCGTC  AATAACCACG  GGAATTTGCG  GACCACGATG  CGGACTTATT

3701   GTGATAATAA  GCGGATGAAT  GGCAGAAATT  CGAAAGCAAA  TTCGACCCGG
       CACTATTATT  CGCCTACTTA  CCGTCTTTAA  GCTTTCGTTT  AAGCTGGGCC

3751   TCGTCGGTTC  AGGGCAGGGT  CGTTAAATAG  CCGCTTATGT  CTATTGCTGG
       AGCAGCCAAG  TCCCGTCCCA  GCAATTTATC  GGCGAATACA  GATAACGACC

3801   TTTACCGGTT  TATTGACTAC  CGGAAGCAGT  GTGACCGTGT  GCTTCTCAAA
       AAATGGCCAA  ATAACTGATG  GCCTTCGTCA  CACTGGCACA  CGAAGAGTTT

3851   TGCCTGAGGC  CAGTTTGCTC  AGGCTCTCCC  CGTGGAGGTA  ATAATTGCTC
       ACGGACTCCG  GTCAAACGAG  TCCGAGAGGG  GCACCTCCAT  TATTAACGAG

3901   GACATGACCA  AAATCCCTTA  ACGTGAGTTT  TCGTTCCACT  GAGCGTCAGA
       CTGTACTGGT  TTTAGGGAAT  TGCACTCAAA  AGCAAGGTGA  CTCGCAGTCT

3951   CCCCGTAGAA  AAGATCAAAG  GATCTTCTTG  AGATCCTTTT  TTTCTGCGCG
       GGGGCATCTT  TTCTAGTTTC  CTAGAAGAAC  TCTAGGAAAA  AAAGACGCGC

4001   TAATCTGCTG  CTTGCAAACA  AAAAAACCAC  CGCTACCAGC  GGTGGTTTGT
       ATTAGACGAC  GAACGTTTGT  TTTTTTGGTG  GCGATGGTCG  CCACCAAACA

4051   TTGCCGGATC  AAGAGCTACC  AACTCTTTTT  CCGAAGGTAA  CTGGCTTCAG
       AACGGCCTAG  TTCTCGATGG  TTGAGAAAAA  GGCTTCCATT  GACCGAAGTC
```

Figure 3: pBS vector series: functional map and sequence of pBS13 (continued)

```
4101    CAGAGCGCAG ATACCAAATA CTGTCCTTCT AGTGTAGCCG TAGTTAGGCC
        GTCTCGCGTC TATGGTTTAT GACAGGAAGA TCACATCGGC ATCAATCCGG

4151    ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC TCTGCTAATC
        TGGTGAAGTT CTTGAGACAT CGTGGCGGAT GTATGGAGCG AGACGATTAG

4201    CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT
        GACAATGGTC ACCGACGACG GTCACCGCTA TTCAGCACAG AATGGCCCAA

4251    GGACTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG
        CCTGAGTTCT GCTATCAATG GCCTATTCCG CGTCGCCAGC CCGACTTGCC

4301    GGGGTTCGTG CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG
        CCCCAAGCAC GTGTGTCGGG TCGAACCTCG CTTGCTGGAT GTGGCTTGAC

4351    AGATACCTAC AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG
        TCTATGGATG TCGCACTCGA TACTCTTTCG CGGTGCGAAG GGCTTCCCTC

4401    AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA GGAGAGCGCA
        TTTCCGCCTG TCCATAGGCC ATTCGCCGTC CCAGCCTTGT CCTCTCGCGT

4451    CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG TCCTGTCGGG
        GCTCCCTCGA AGGTCCCCCT TTGCGGACCA TAGAAATATC AGGACAGCCC

4501    TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG
        AAAGCGGTGG AGACTGAACT CGCAGCTAAA AACACTACGA GCAGTCCCCC

4551    GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG
        CGCCTCGGAT ACCTTTTTGC GGTCGTTGCG CCGGAAAAAT GCCAAGGACC

4601    CCTTTTGCTG GCCTTTTGCT CACATG
        GGAAAACGAC CGGAAAACGA GTGTAC
```

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Figure 4: Co-existence of phagemids: results of restriction digest

R1
+
R1 R2 R2 M1 1 2 3 4 5 M2 6 7 8 9 10
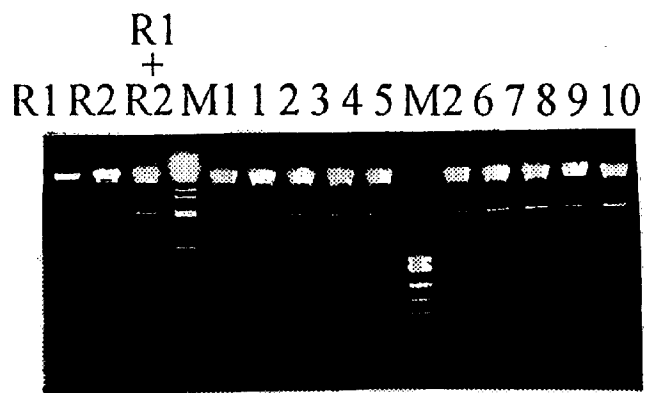
Figure 5: Phagemid vector pYING1-C1: functional map
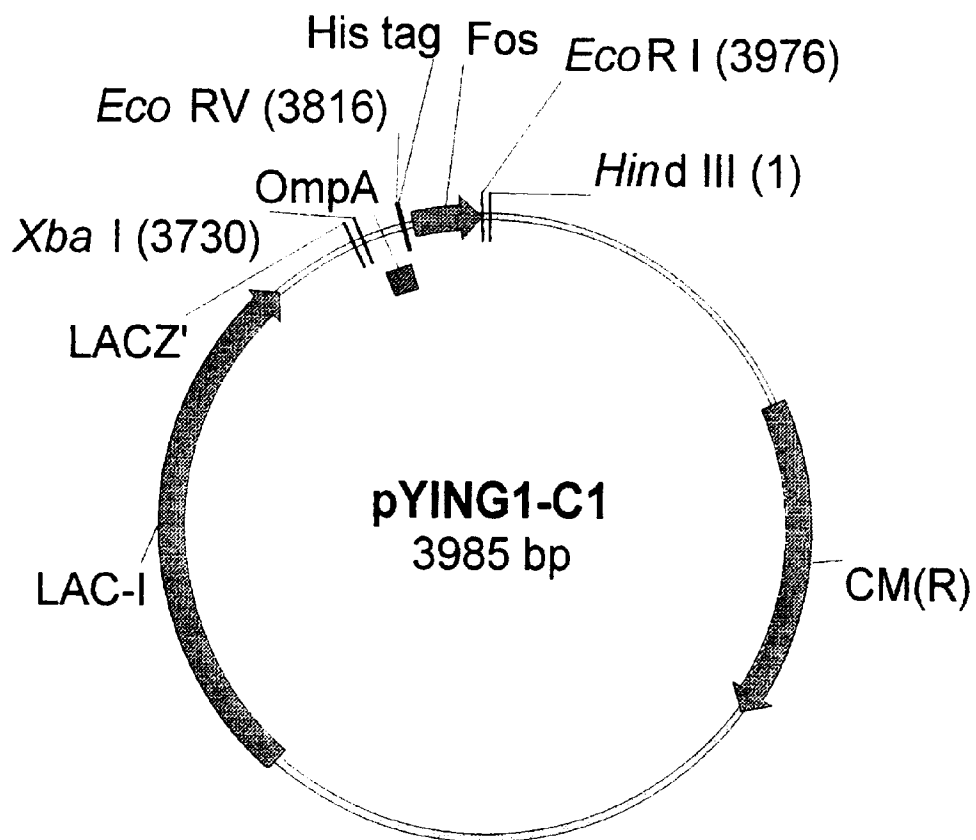
Figure 6: Phagemid vector pYANG3-A: functional map
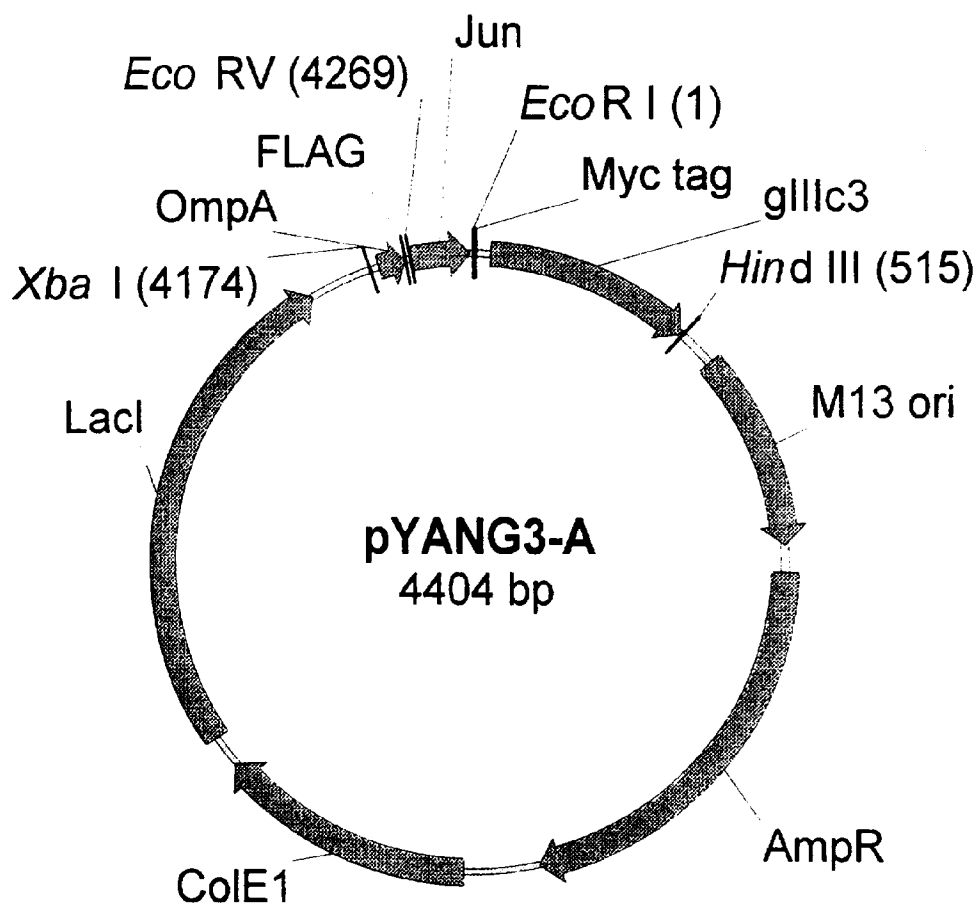
Figure 7: Analysis of selected clones (see Table 2)

7.a: Restriction digest of clones before and after selection

α   β
Before selection   After selection

R M1   M2
1234567891 0   1234567891 0

Pep-gIII
Jun-gIII
p75

Fos

7.b: PCR of clones after selection with primers OPEP5L and OGIII3
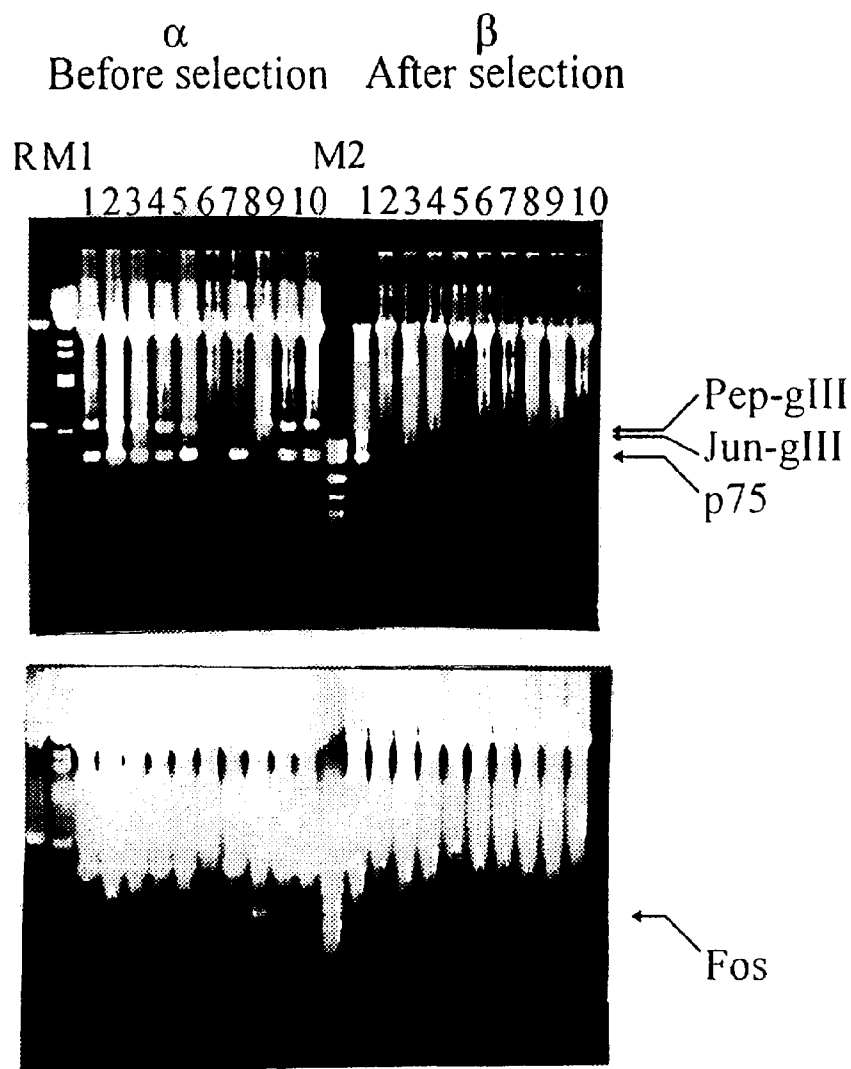
β: after selection
R1R2M12345678910
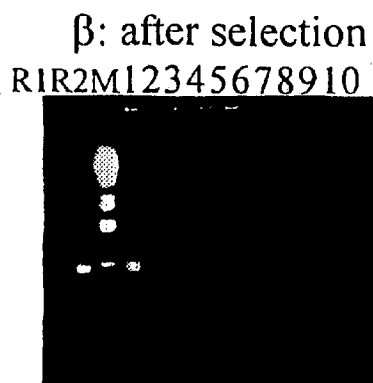
Figure 8: Phagemid vector pING1-C1: functional map
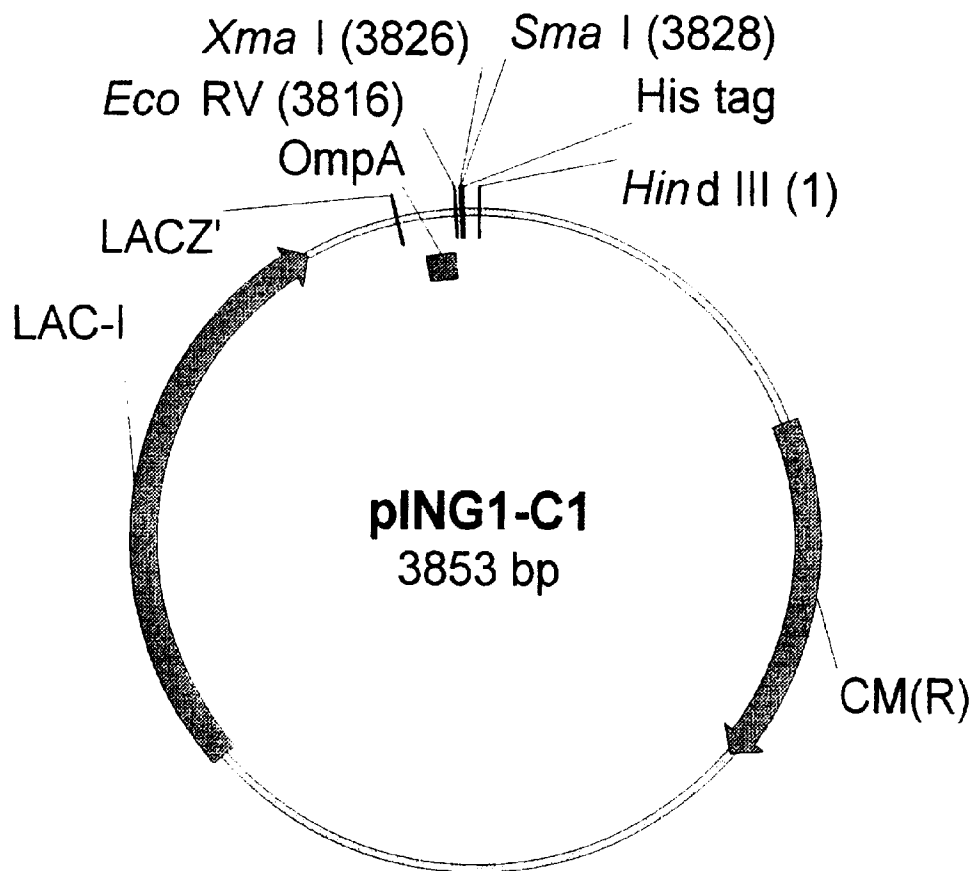
Figure 9: Phagemid vector pONG3-A: functional map
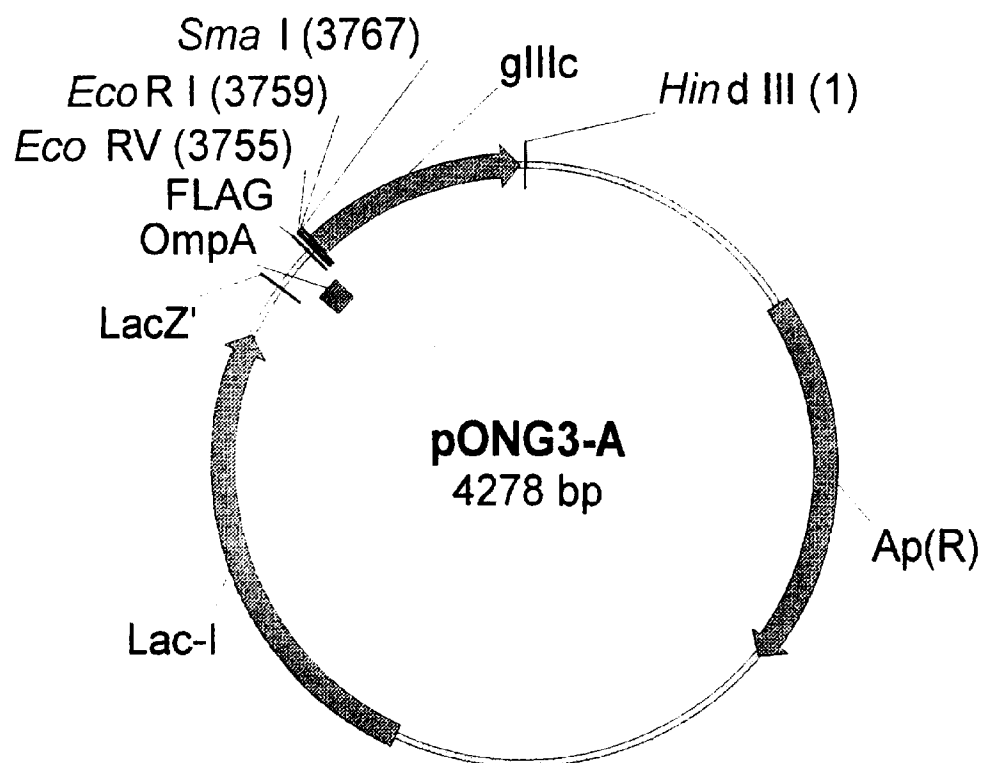
Figure 10: Co-transformation of phage and plasmid, polyphage formation and selection *via* SIP: general description

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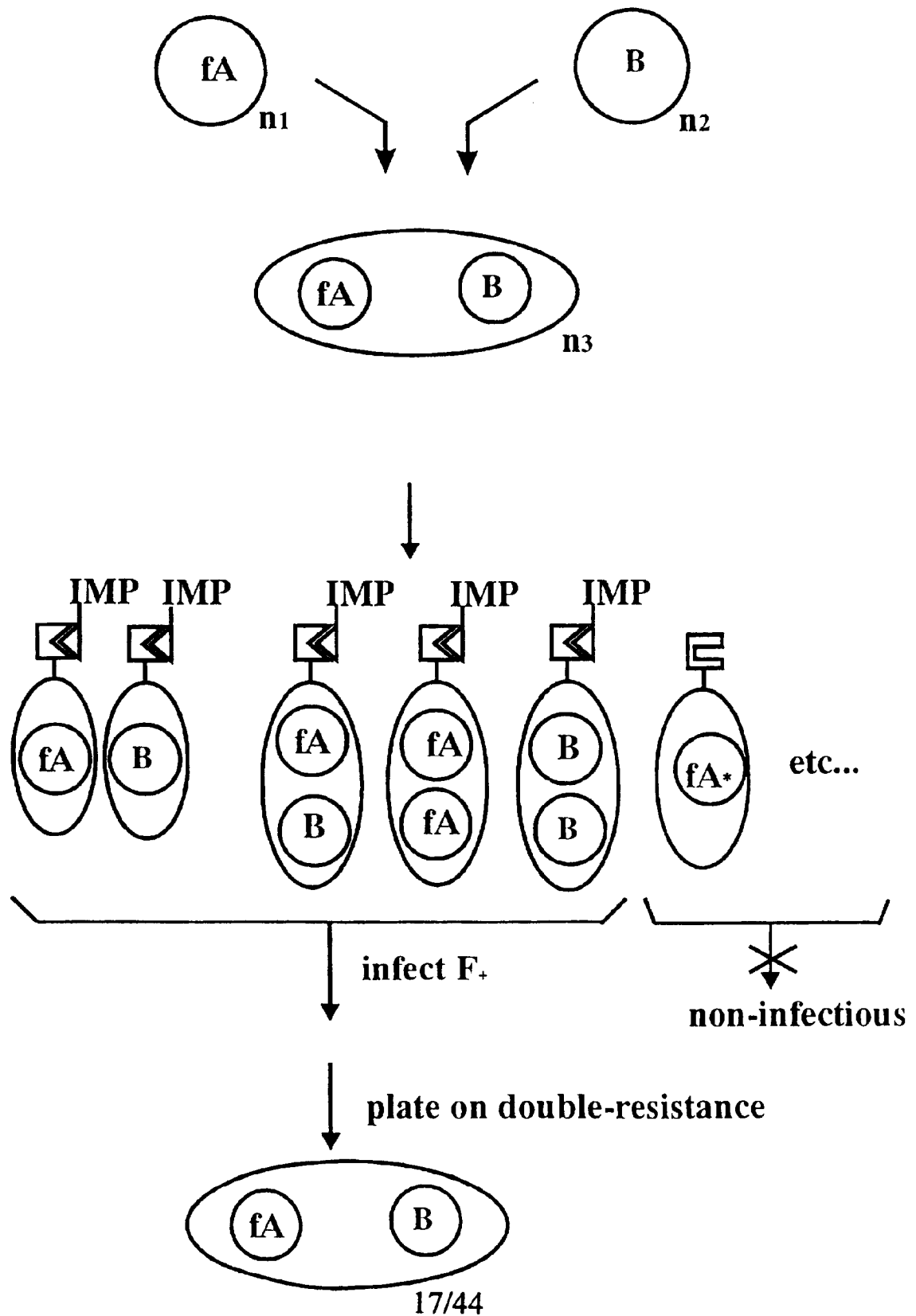
Figure 11: Phage vector fhag1A: functional map

Figure 11a: CAT gene module: functional map and sequence

813 bp
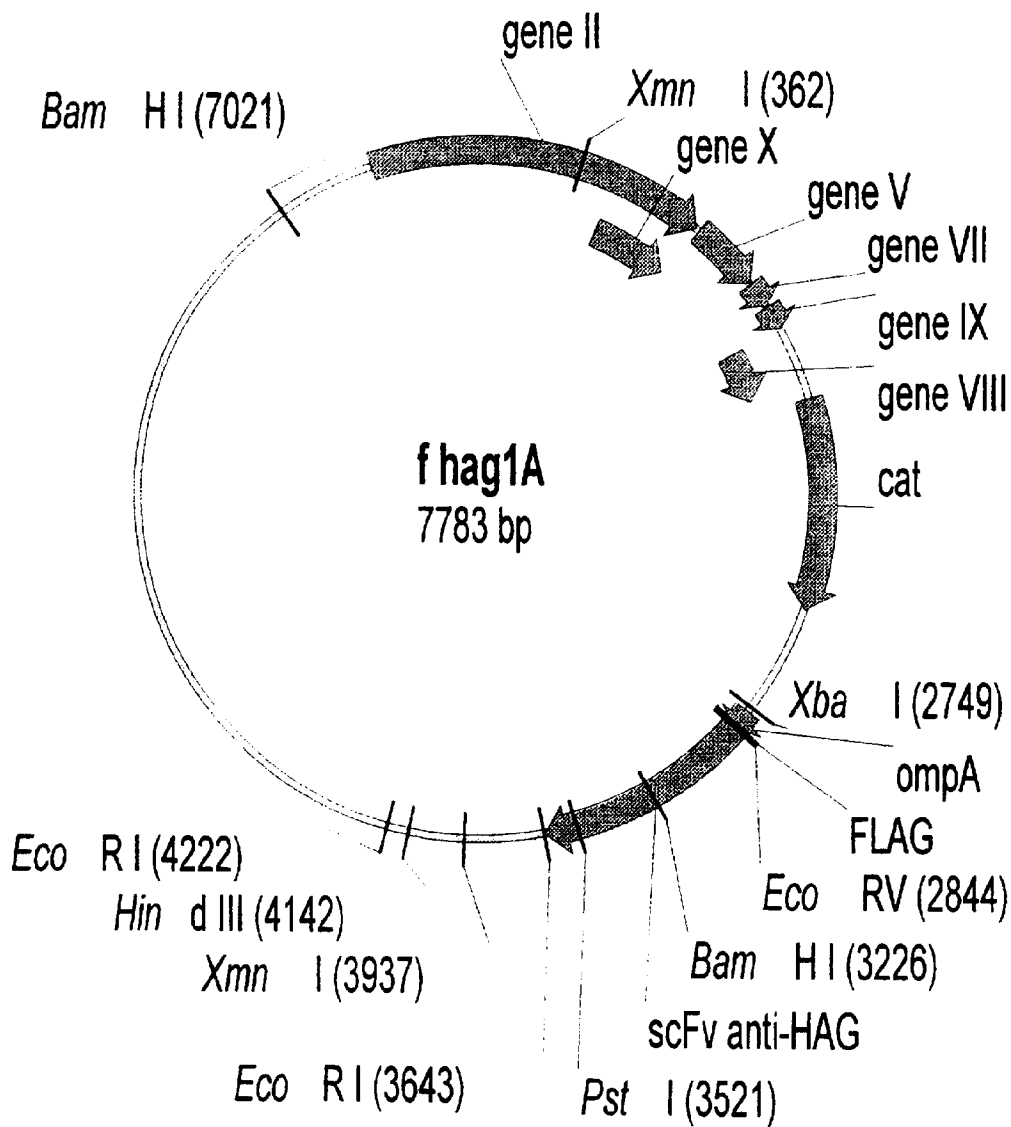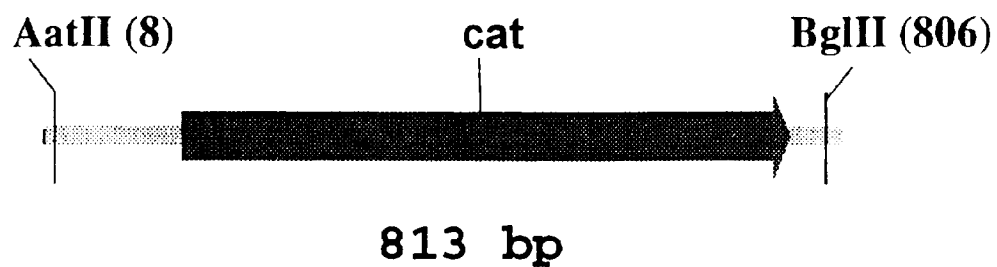
Figure 11a: CAT gene module: functional map and sequence (cont.)

```
          AatII
          ~~~~~~
    1   GGGACGTCGG GTGAGGTTCC AACTTTCACC ATAATGAAAT AAGATCACTA
        CCCTGCAGCC CACTCCAAGG TTGAAAGTGG TATTACTTTA TTCTAGTGAT

   51   CCGGGCGTAT TTTTTGAGTT ATCGAGATTT TCAGGAGCTA AGGAAGCTAA
        GGCCCGCATA AAAAACTCAA TAGCTCTAAA AGTCCTCGAT TCCTTCGATT

  101   AATGGAGAAA AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC
        TTACCTCTTT TTTTAGTGAC CTATATGGTG GCAACTATAT AGGGTTACCG

  151   ATCGTAAAGA ACATTTTGAG GCATTTCAGT CAGTTGCTCA ATGTACCTAT
        TAGCATTTCT TGTAAAACTC CGTAAAGTCA GTCAACGAGT TACATGGATA

  201   AACCAGACCG TTCAGCTGGA TATTACGGCC TTTTTAAAGA CCGTAAAGAA
        TTGGTCTGGC AAGTCGACCT ATAATGCCGG AAAAATTTCT GGCATTTCTT

  251   AAATAAGCAC AAGTTTTATC CGGCCTTTAT TCACATTCTT GCCCGCCTGA
        TTTATTCGTG TTCAAAATAG GCCGGAAATA AGTGTAAGAA CGGGCGGACT

  301   TGAATGCTCA CCCGGAGTTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG
        ACTTACGAGT GGGCCTCAAG GCATACCGTT ACTTTCTGCC ACTCGACCAC

  351   ATATGGGATA GTGTTCACCC TTGTTACACC GTTTTCCATG AGCAAACTGA
        TATACCCTAT CACAAGTGGG AACAATGTGG CAAAAGGTAC TCGTTTGACT

  401   AACGTTTTCA TCGCTCTGGA GTGAATACCA CGACGATTTC CGGCAGTTTC
        TTGCAAAAGT AGCGAGACCT CACTTATGGT GCTGCTAAAG GCCGTCAAAG

  451   TACACATATA TTCGCAAGAT GTGGCGTGTT ACGGTGAAAA CCTGGCCTAT
        ATGTGTATAT AAGCGTTCTA CACCGCACAA TGCCACTTTT GGACCGGATA

  501   TTCCCTAAAG GGTTTATTGA GAATATGTTT TTCGTCTCAG CCAATCCCTG
        AAGGGATTTC CCAAATAACT CTTATACAAA AAGCAGAGTC GGTTAGGGAC

  551   GGTGAGTTTC ACCAGTTTTG ATTTAAACGT AGCCAATATG GACAACTTCT
        CCACTCAAAG TGGTCAAAAC TAAATTTGCA TCGGTTATAC CTGTTGAAGA

  601   TCGCCCCCGT TTTCACTATG GGCAAATATT ATACGCAAGG CGACAAGGTG
        AGCGGGGGCA AAAGTGATAC CCGTTTATAA TATGCGTTCC GCTGTTCCAC

  651   CTGATGCCGC TGGCGATTCA GGTTCATCAT GCCGTTTGTG ATGGCTTCCA
        GACTACGGCG ACCGCTAAGT CCAAGTAGTA CGGCAAACAC TACCGAAGGT

  701   TGTCGGCAGA ATGCTTAATG AATTACAACA GTACTGCGAT GAGTGGCAGG
        ACAGCCGTCT TACGAATTAC TTAATGTTGT CATGACGCTA CTCACCGTCC

  751   GCGGGGCGTA ATTTTTTTAA GGCAGTTATT GGGTGCCCTT AAACGCCTGG
        CGCCCCGCAT TAAAAAAATT CCGTCAATAA CCCACGGGAA TTTGCGGACC

          BglII
          ~~~~~~
  801   TGCTAGATCT TCC
        ACGATCTAGA AGG
```

Figure 12: Phage vector fjun1A: functional map

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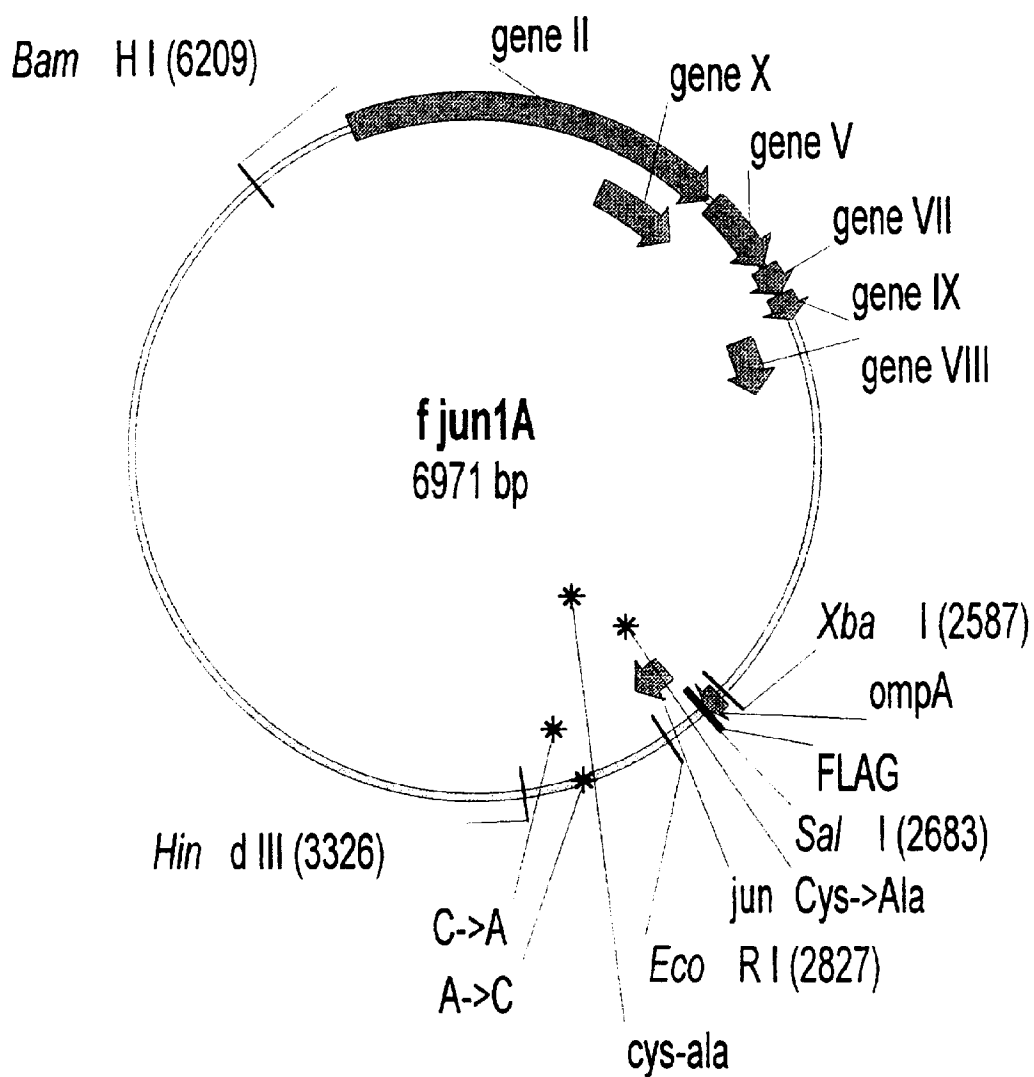
Figure 13: Phage vector fjun1B: functional map
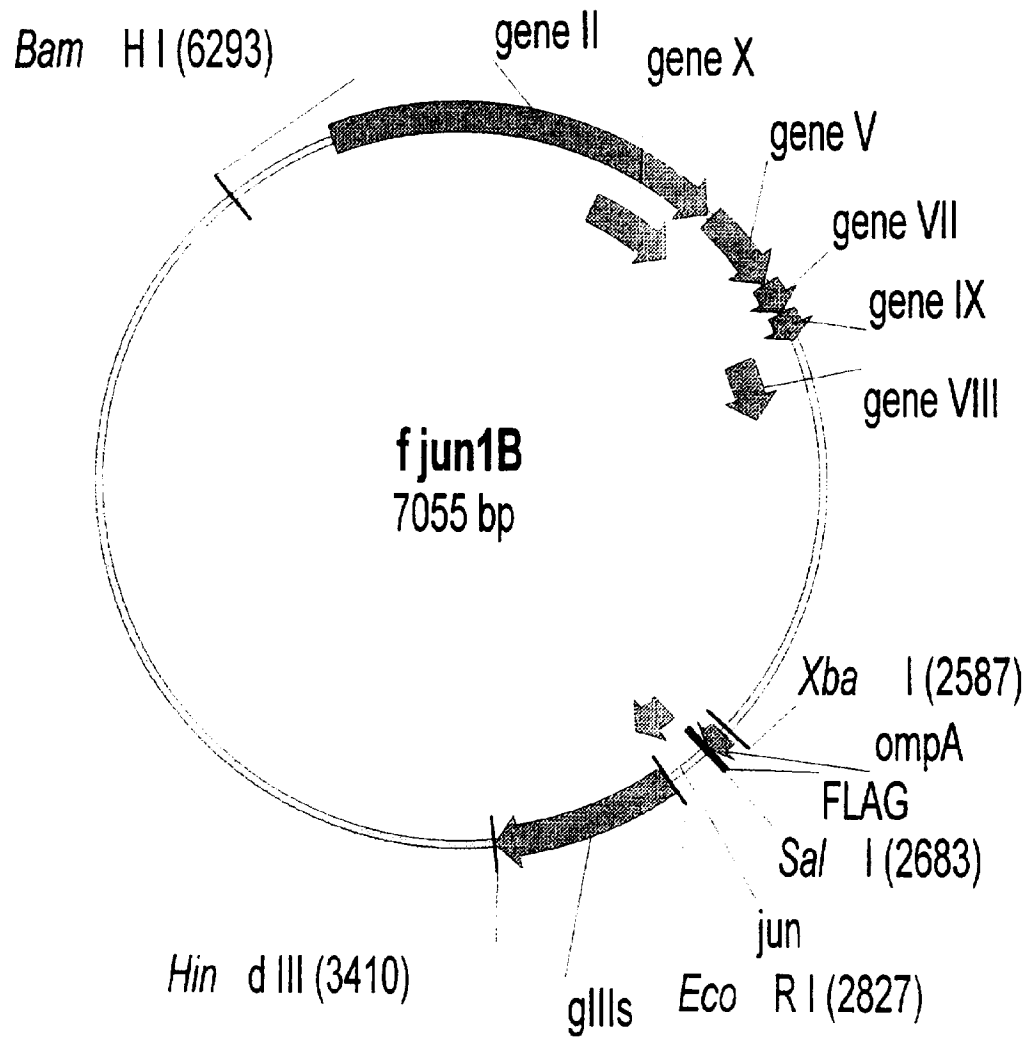
Figure 14: Phage vector fpep3_1B: functional map
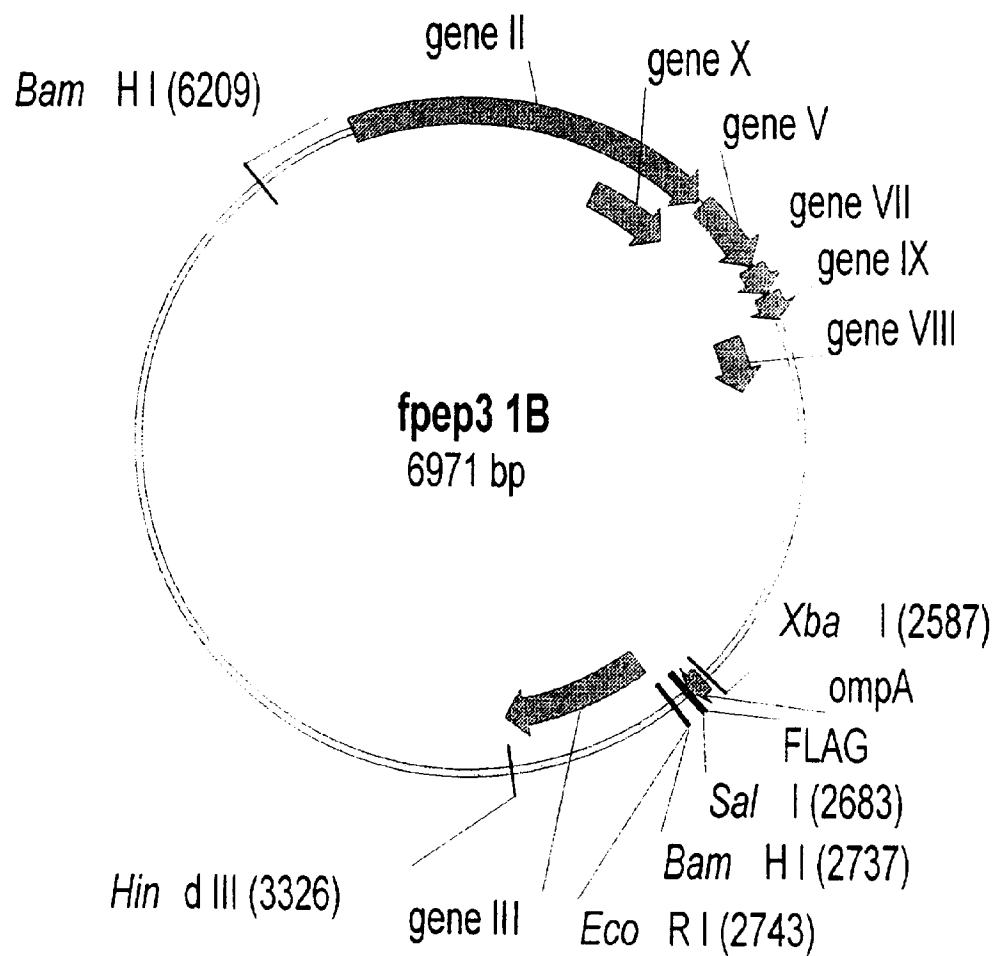
Figure 15: Phage vector fNGF_1B: functional map
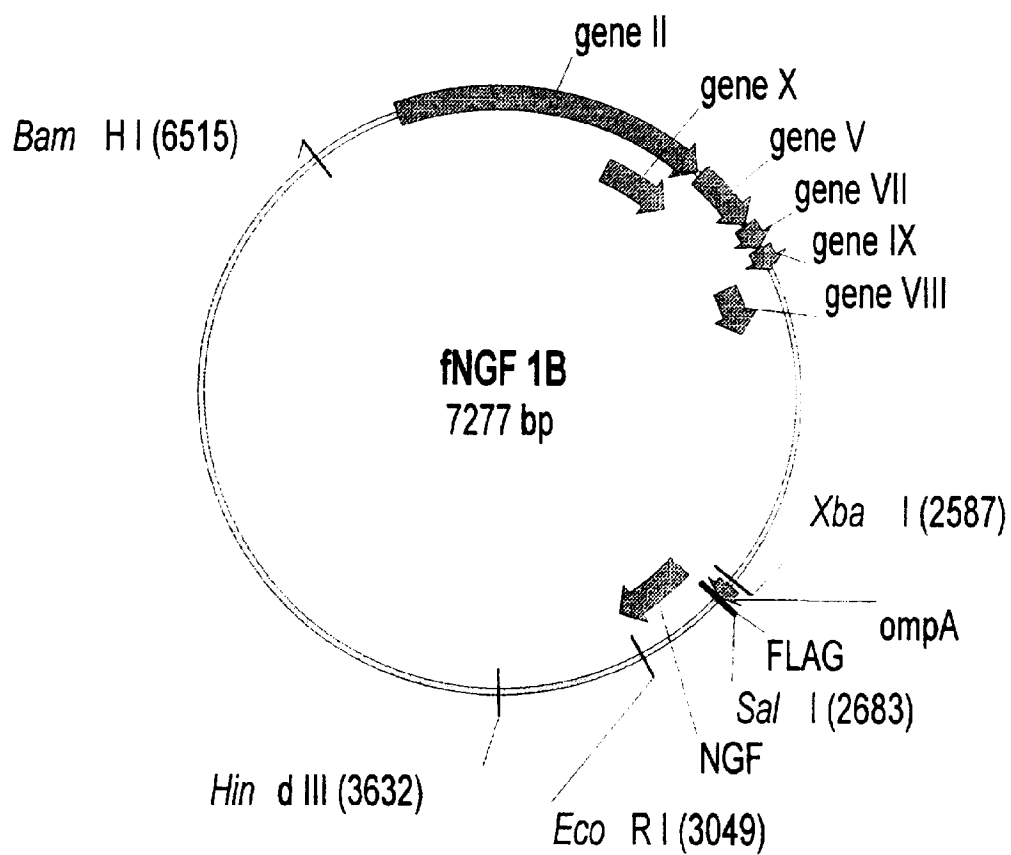
Figure 16: Plasmid pUC19/IMPhag: functional map
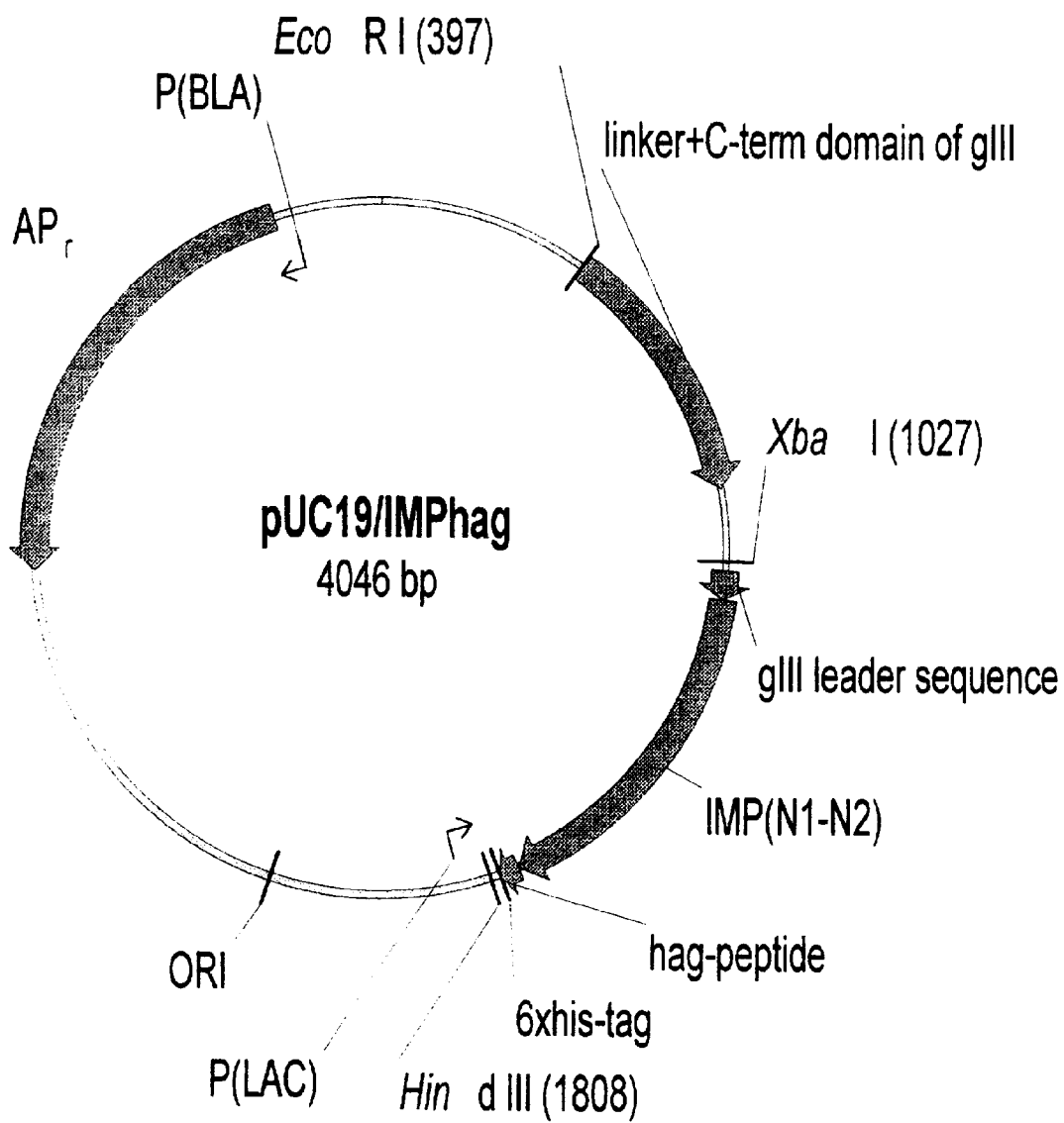
Figure 17: Plasmid pUC18/IMPp75: functional map
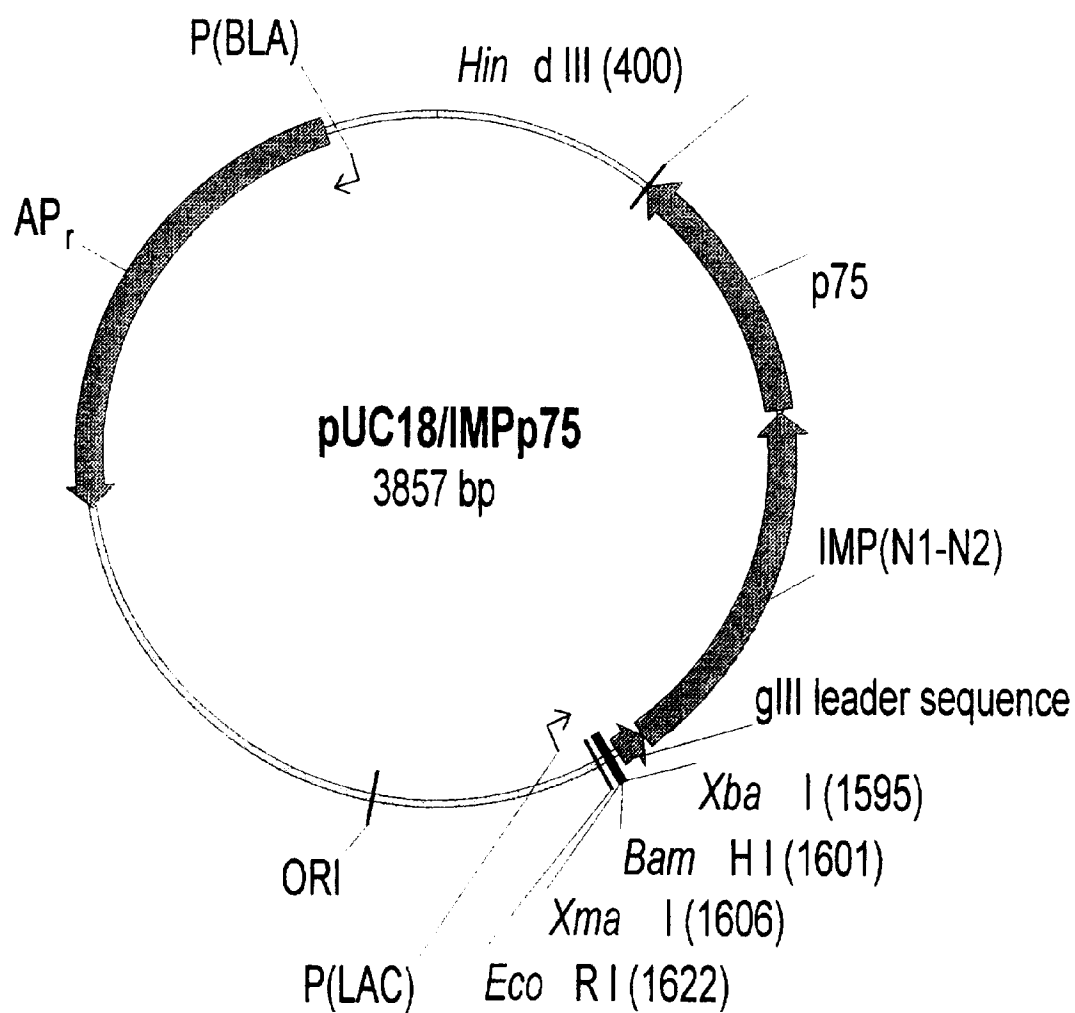
Figure 18: Plasmid pUC18/IMPIL16: functional map
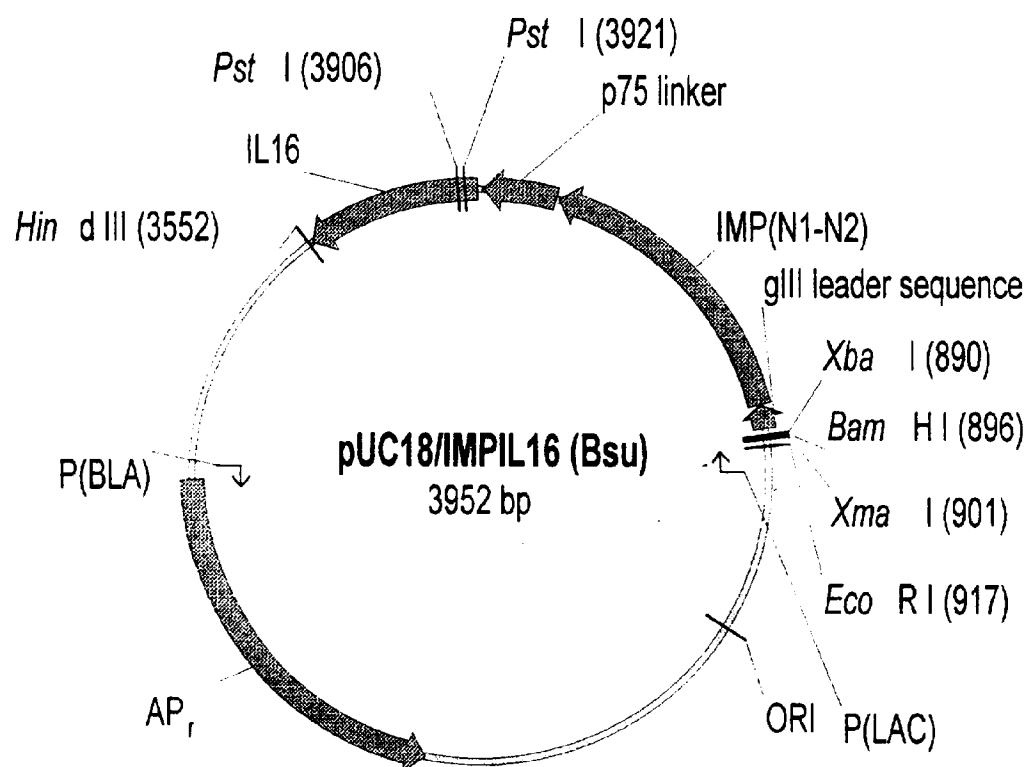
Figure 19: Analysis of selected clones (see Table 3)
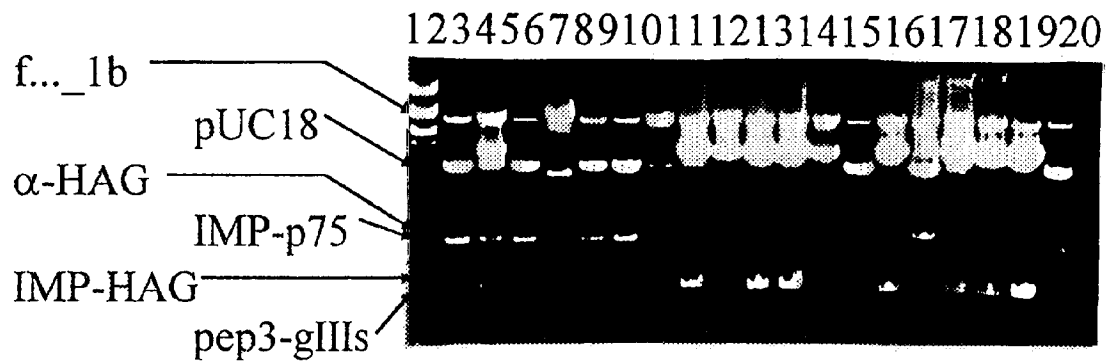
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

f..._1b

pUC18

α-HAG

IMP-p75

IMP-HAG

pep3-gIIIs

Figure 20: Co-transformation of phagemids, *in vivo* recombination and selection *via* His-tag: general description
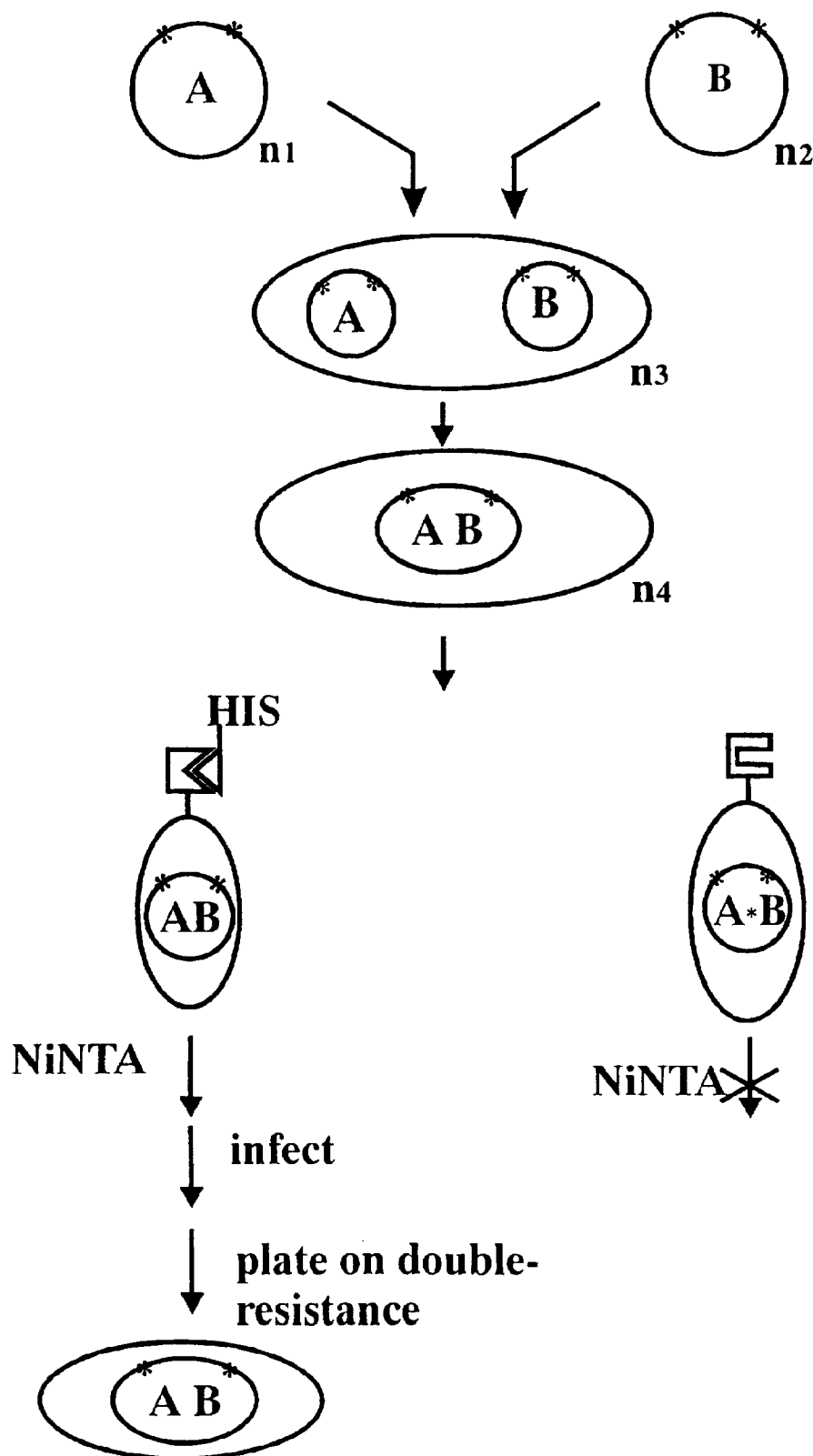
Figure 21: *In vitro* recombination and selection *via* His-tag:
general description
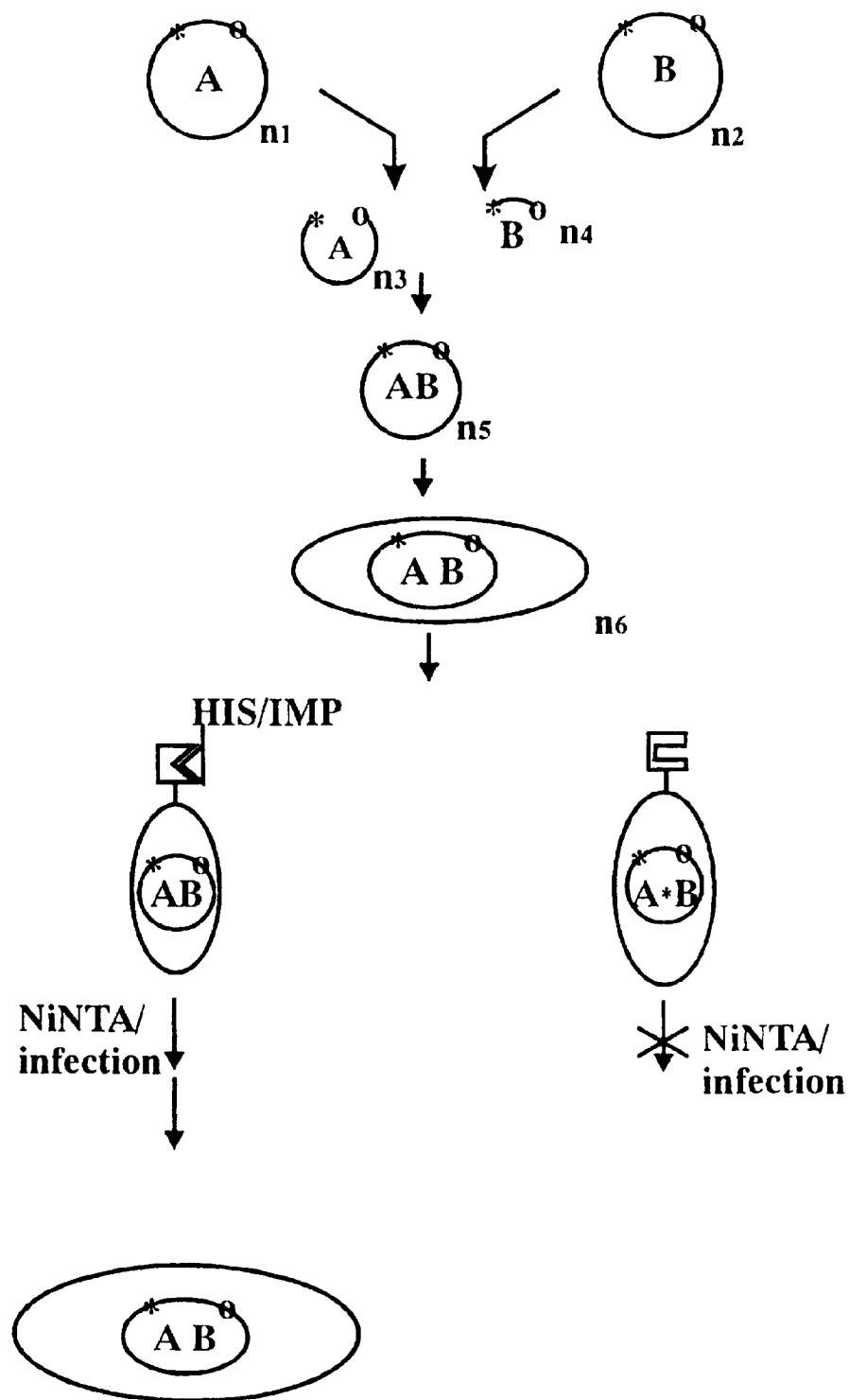
Figure 22: Phage vector fjunhag: functional map
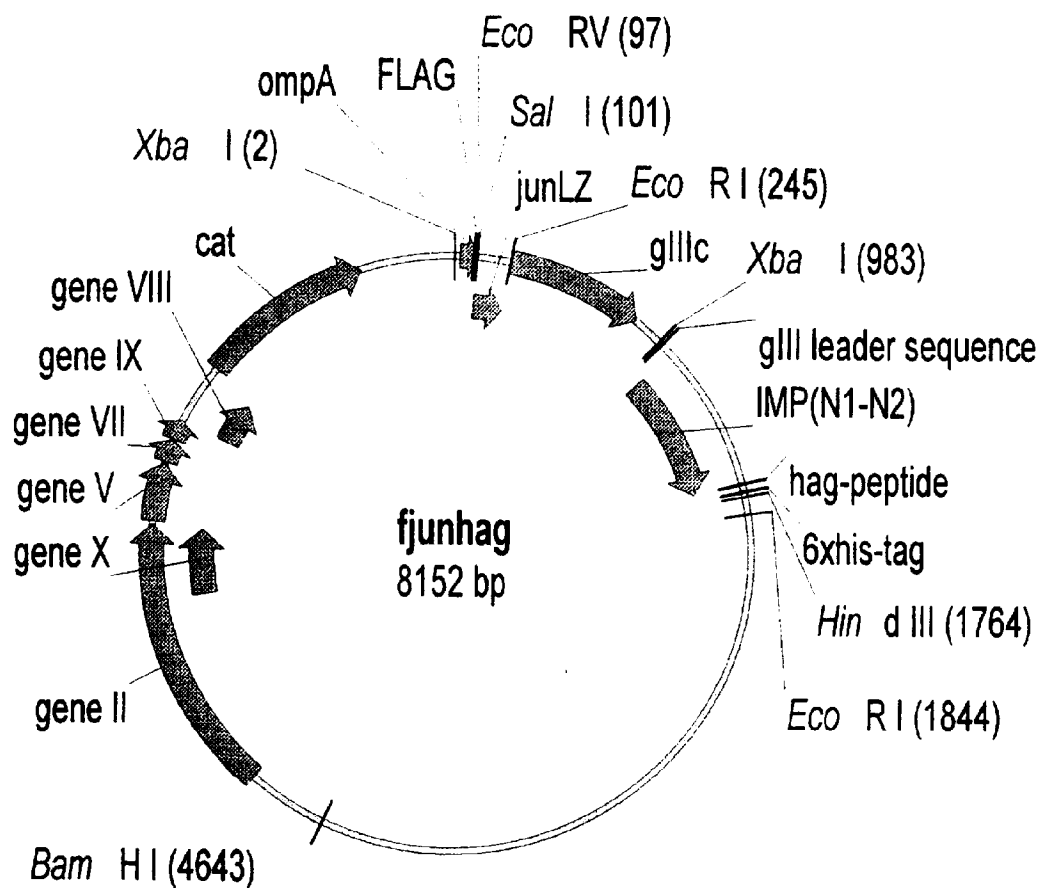
Figure 23: Spatial *in vivo* SIP: general description

analysis of individual clones

master plate

information

screen for tag/ infectivity

replica
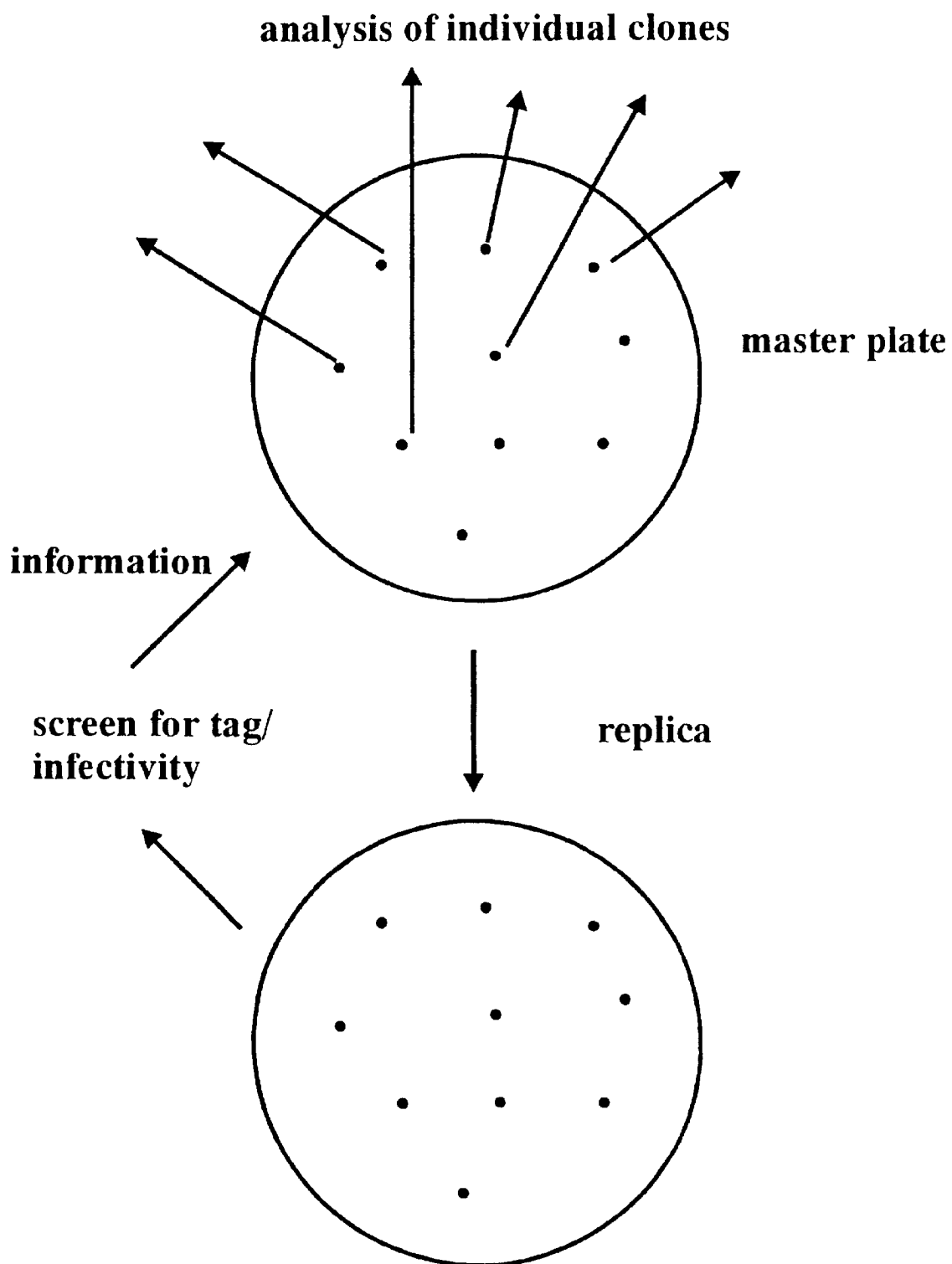
Figure 24: *E. coli* display: general description
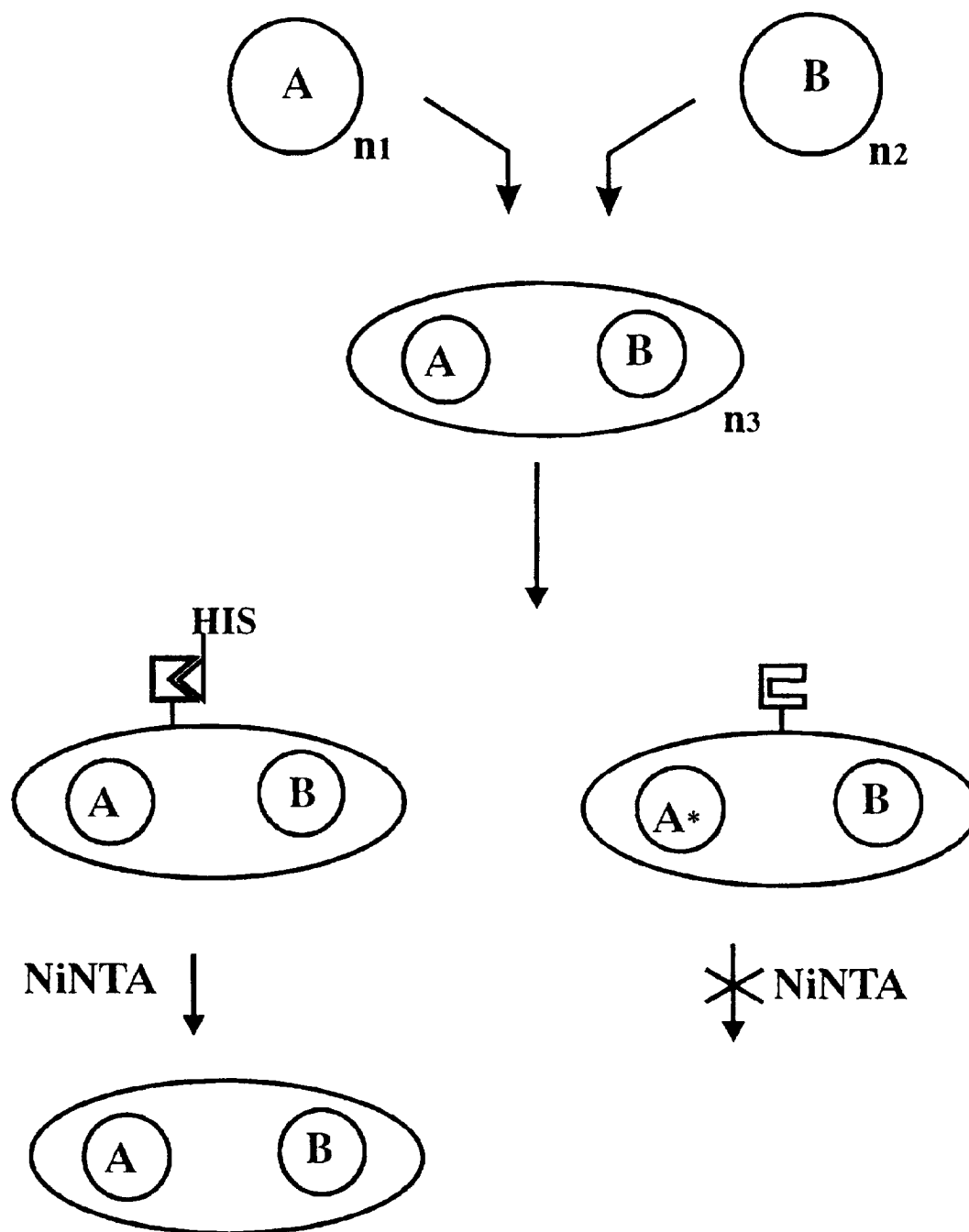
Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence
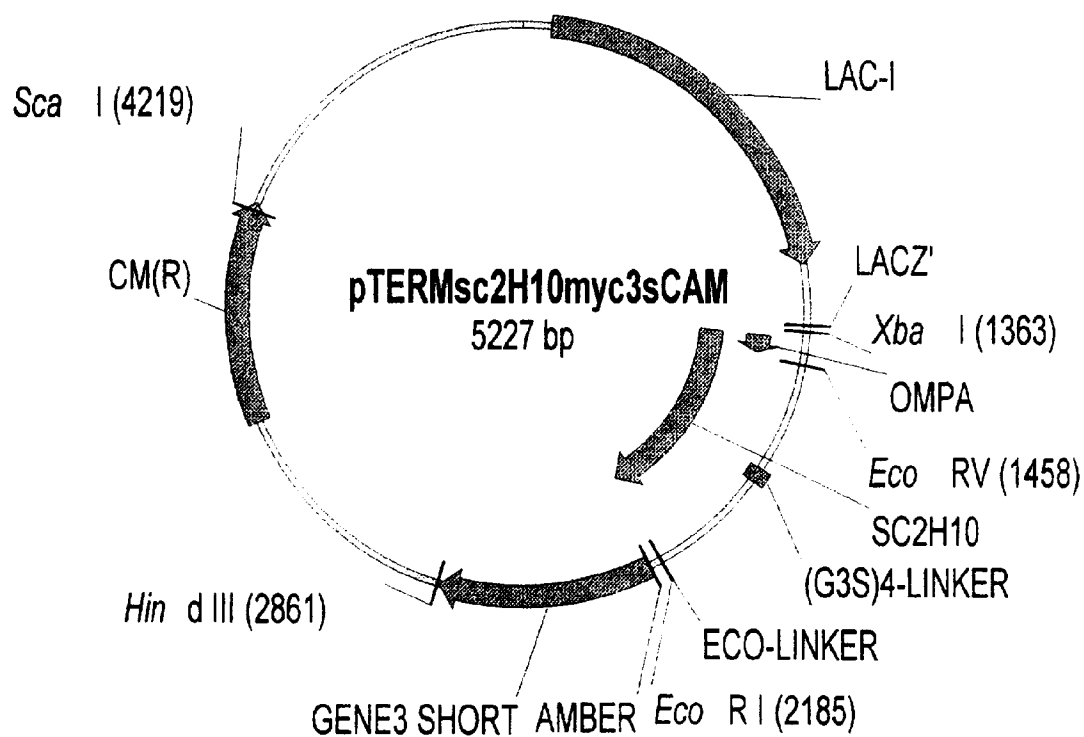
Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
  1  ACCCGACACC ATCGAATGGC GCAAAACCTT TCGCGGTATG GCATGATAGC
     TGGGCTGTGG TAGCTTACCG CGTTTTGGAA AGCGCCATAC CGTACTATCG

 51  GCCCGGAAGA GAGTCAATTC AGGGTGGTGA ATGTGAAACC AGTAACGTTA
     CGGGCCTTCT CTCAGTTAAG TCCCACCACT TACACTTTGG TCATTGCAAT

101  TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG TTTCCCGCGT
     ATGCTACAGC GTCTCATACG GCCACAGAGA ATAGTCTGGC AAAGGGCGCA

151  GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG
     CCACTTGGTC CGGTCGGTGC AAAGACGCTT TTGCGCCCTT TTTCACCTTC

201  CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACTG
     GCCGCTACCG CCTCGACTTA ATGTAAGGGT TGGCGCACCG TGTTGTTGAC

251  GCGGGCAAAC AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT
     CGCCCGTTTG TCAGCAACGA CTAACCGCAA CGGTGGAGGT CAGACCGGGA

301  GCACGCGCCG TCGCAAATTG TCGCGGCGAT TAAATCTCGC GCCGATCAAC
     CGTGCGCGGC AGCGTTTAAC AGCGCCGCTA ATTTAGAGCG CGGCTAGTTG

351  TGGGTGCCAG CGTGGTGGTG TCGATGGTAG AACGAAGCGG CGTCGAAGCC
     ACCCACGGTC GCACCACCAC AGCTACCATC TTGCTTCGCC GCAGCTTCGG

401  TGTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA GTGGGCTGAT
     ACATTTCGCC GCCACGTGTT AGAAGAGCGC GTTGCGCAGT CACCCGACTA

451  CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
     GTAATTGATA GGCGACCTAC TGGTCCTACG GTAACGACAC CTTCGACGGA

501  GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC
     CGTGATTACA AGGCCGCAAT AAAGAACTAC AGAGACTGGT CTGTGGGTAG

551  AACAGTATTA TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA
     TTGTCATAAT AAAAGAGGGT ACTTCTGCCA TGCGCTGACC CGCACCTCGT

601  TCTGGTCGCA TTGGGTCACC AGCAAATCGC GCTGTTAGCG GGCCCATTAA
     AGACCAGCGT AACCCAGTGG TCGTTTAGCG CGACAATCGC CCGGGTAATT

651  GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG GCTGGCATAA ATATCTCACT
     CAAGACAGAG CCGCGCAGAC GCAGACCGAC CGACCGTATT TATAGAGTGA

701  CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT GGAGTGCCAT
     GCGTTAGTTT AAGTCGGCTA TCGCCTTGCC CTTCCGCTGA CCTCACGGTA

751  GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA
     CAGGCCAAAA GTTGTTTGGT ACGTTTACGA CTTACTCCCG TAGCAAGGGT

801  CTGCGATGCT GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC
     GACGCTACGA CCAACGGTTG CTAGTCTACC GCGACCCGCG TTACGCGCGG
```

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Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
 851   ATTACCGAGT CCGGGCTGCG CGTTGGTGCG GACATCTCGG TAGTGGGATA
       TAATGGCTCA GGCCCGACGC GCAACCACGC CTGTAGAGCC ATCACCCTAT

 901   CGACGATACC GAAGACAGCT CATGTTATAT CCCGCCGTTA ACCACCATCA
       GCTGCTATGG CTTCTGTCGA GTACAATATA GGGCGGCAAT TGGTGGTAGT

 951   AACAGGATTT TCGCCTGCTG GGGCAAACCA GCGTGGACCG CTTGCTGCAA
       TTGTCCTAAA AGCGGACGAC CCCGTTTGGT CGCACCTGGC GAACGACGTT

1001   CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC CCGTCTCACT
       GAGAGAGTCC CGGTCCGCCA CTTCCCGTTA GTCGACAACG GGCAGAGTGA

1051   GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC
       CCACTTTTCT TTTTGGTGGG ACCGCGGGTT ATGCGTTTGG CGGAGAGGGG

1101   GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG
       CGCGCAACCG GCTAAGTAAT TACGTCGACC GTGCTGTCCA AAGGGCTGAC

1151   GAAAGCGGGC AGTGAGCGGT ACCCGATAAA AGCGGCTTCC TGACAGGAGG
       CTTTCGCCCG TCACTCGCCA TGGGCTATTT TCGCCGAAGG ACTGTCCTCC

1201   CCGTTTTGTT TTGCAGCCCA CCTCAACGCA ATTAATGTGA GTTAGCTCAC
       GGCAAAACAA AACGTCGGGT GGAGTTGCGT TAATTACACT CAATCGAGTG

1251   TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT
       AGTAATCCGT GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA

1301   GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT
       CACCTTAACA CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GATACTGGTA

                          Xba I
                       ~~~~~~
1351   GATTACGAAT TCTAGATAA CGAGGGCAAA AAATGAAAAA GACAGCTATC
       CTAATGCTTA AAGATCTATT GCTCCCGTTT TTTACTTTTT CTGTCGATAG

1401   GCGATTGCAG TGGCACTGGC TGGTTTCGCT ACCGTAGCGC AGGCCGACTA
       CGCTAACGTC ACCGTGACCG ACCAAAGCGA TGGCATCGCG TCCGGCTGAT

                          EcoRV
                       ~~~~~~
1451   CAAAGATATC GTGATGACCC AGTCTCCAGC AATCATGTCT ACATCTCTAG
       GTTTCTATAG CACTACTGGG TCAGAGGTCG TTAGTACAGA TGTAGAGATC

1501   GGGAACGGGT CACCATGACC TGCACTGCCA GTTCAAGTGT AAGTTCCTCT
       CCCTTGCCCA GTGGTACTGG ACGTGACGGT CAAGTTCACA TTCAAGGAGA

1551   TACTTACACT GGTACCAGCA GAAGCCAGGA TCCTCCCCCA AACTCTGGAT
       ATGAATGTGA CCATGGTCGT CTTCGGTCCT AGGAGGGGGT TTGAGACCTA

1601   TTATAGCACA TCCAACCTGG CTTCTGGAGT CCCAACTCGC TTCAGTGGCA
```

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Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
          AATATCGTGT AGGTTGGACC GAAGACCTCA GGGTTGAGCG AAGTCACCGT

1651      GTGGGTCTGG GACCTCTTAC TCTCTCACAA TCAGCACCAT GGCGGCTGAG
          CACCCAGACC CTGGAGAATG AGAGAGTGTT AGTCGTGGTA CCGCCGACTC

1701      GATGCTGCCA CTTATTACTG CCACCAGTAT CATCGTTTCC CACCCACGTT
          CTACGACGGT GAATAATGAC GGTGGTCATA GTAGCAAAGG GTGGGTGCAA

1751      CGGAGGGGGG ACCAAGCTGG AAATAAAACG GGCTGGTGGT GGTGGTTCTG
          GCCTCCCCCC TGGTTCGACC TTTATTTTGC CCGACCACCA CCACCAAGAC

1801      GCGGCGGCGG CTCCGGTGGT GGTGGTTCTG AAGTTAAACT GGTCGAGTCT
          CGCCGCCGCC GAGGCCACCA CCACCAAGAC TTCAATTTGA CCAGCTCAGA

1851      GGAGGAGGCT TGGTGCAACC TGGAGGATCC ATGAAACTCT CCTGTGTTGC
          CCTCCTCCGA ACCACGTTGG ACCTCCTAGG TACTTTGAGA GGACACAACG

1901      CTCTGGAATC ACTTTCAGTA ATTACCGGAT GAACTGGGTC CGCCAGTCTC
          GAGACCTTAG TGAAAGTCAT TAATGGCCTA CTTGACCCAG GCGGTCAGAG

1951      CAGAGAAGGG GCTTGAGTGG GTTGCTGAAA TTAGATTGAA ATCTAATAAT
          GTCTCTTCCC CGAACTCACC CAACGACTTT AATCTAACTT TAGATTATTA

2001      TATGCAACAC ATTATGCGGA GTCTGTGAAA GGGAGGTTCA CCATCTCAAG
          ATACGTTGTG TAATACGCCT CAGACACTTT CCCTCCAAGT GGTAGAGTTC

2051      AGATGATTCC AAAAGTAGTG TCTACCTGCA AATGAACAAC TTAAGAGCTG
          TCTACTAAGG TTTTCATCAC AGATGGACGT TTACTTGTTG AATTCTCGAC

2101      AAGACACTGG CATTTATTAC TGTAGAGGGG TTTCATATAC TATAGACTAC
          TTCTGTGACC GTAAATAATG ACATCTCCCC AAAGTATATG ATATCTGATG

                                              EcoRI
                                              ~~~~~~
2151      TGGGGTCAAG GAACCTCAGT CACAGTCTCC TCAGAATTCG AGCAGAAGCT
          ACCCCAGTTC CTTGGAGTCA GTGTCAGAGG AGTCTTAAGC TCGTCTTCGA

2201      GATCTCTGAG GAAGACCTGT AGGCATGCTT ATTTGTTTGT GAATATCAAG
          CTAGAGACTC CTTCTGGACA TCCGTACGAA TAAACAAACA CTTATAGTTC

2251      GCCAATCGTC TGACCTGCCT CAACCTCCTG TCAATGCTGG CGGCGGCTCT
          CGGTTAGCAG ACTGGACGGA GTTGGAGGAC AGTTACGACC GCCGCCGAGA

2301      GGTGGTGGTT CTGGTGGCGG CTCTGAGGGT GGTGGCTCTG AGGGTGGCGG
          CCACCACCAA GACCACCGCC GAGACTCCCA CCACCGAGAC TCCCACCGCC

2351      TTCTGAGGGT GGCGGCTCTG AGGGAGGCGG TTCCGGTGGT GGCTCTGGTT
          AAGACTCCCA CCGCCGAGAC TCCCTCCGCC AAGGCCACCA CCGAGACCAA

2401      CCGGTGATTT TGATTATGAA AAGATGGCAA ACGCTAATAA GGGGGCTATG
          GGCCACTAAA ACTAATACTT TTCTACCGTT TGCGATTATT CCCCCGATAC
```

Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
2451   ACCGAAAATG CCGATGAAAA CGCGCTACAG TCTGACGCTA AAGGCAAACT
       TGGCTTTTAC GGCTACTTTT GCGCGATGTC AGACTGCGAT TTCCGTTTGA

2501   TGATTCTGTC GCTACTGATT ACGGTGCTGC TATCGATGGT TTCATTGGTG
       ACTAAGACAG CGATGACTAA TGCCACGACG ATAGCTACCA AAGTAACCAC

2551   ACGTTTCCGG CCTTGCTAAT GGTAATGGTG CTACTGGTGA TTTTGCTGGC
       TGCAAAGGCC GGAACGATTA CCATTACCAC GATGACCACT AAAACGACCG

2601   TCTAATTCCC AAATGGCTCA AGTCGGTGAC GGTGATAATT CACCTTTAAT
       AGATTAAGGG TTTACCGAGT TCAGCCACTG CCACTATTAA GTGGAAATTA

2651   GAATAATTTC CGTCAATATT TACCTTCCCT CCCTCAATCG GTTGAATGTC
       CTTATTAAAG GCAGTTATAA ATGGAAGGGA GGGAGTTAGC CAACTTACAG

2701   GCCCTTTTGT CTTTGGCGCT GGTAAACCAT ATGAATTTTC TATTGATTGT
       CGGGAAAACA GAAACCGCGA CCATTTGGTA TACTTAAAAG ATAACTAACA

2751   GACAAAATAA ACTTATTCCG TGGTGTCTTT GCGTTTCTTT TATATGTTGC
       CTGTTTTATT TGAATAAGGC ACCACAGAAA CGCAAAGAAA ATATACAACG

2801   CACCTTTATG TATGTATTTT CTACGTTTGC TAACATACTG CGTAATAAGG
       GTGGAAATAC ATACATAAAA GATGCAAACG ATTGTATGAC GCATTATTCC

                 HindIII
                 ~~~~~~~
2851   AGTCTTGATA AGCTTGACCT GTGAAGTGAA AAATGGCGCA CATTGTGCGA
       TCAGAACTAT TCGAACTGGA CACTTCACTT TTTACCGCGT GTAACACGCT

2901   CATTTTTTTT GTCTGCCGTT TACCGCTACT GCGTCACGGA TCCCCACGCG
       GTAAAAAAAA CAGACGGCAA ATGGCGATGA CGCAGTGCCT AGGGGTGCGC

2951   CCCTGTAGCG GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT
       GGGACATCGC CGCGTAATTC GCGCCGCCCA CACCACCAAT GCGCGTCGCA

3001   GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC
       CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG

3051   CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG
       GAAGGAAAGA GCGGTGCAAG CGGCCGAAAG GGGCAGTTCG AGATTTAGCC

3101   GGCATCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA
       CCGTAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT

3151   AAAACTTGAT TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA
       TTTTGAACTA ATCCCACTAC CAAGTGCATC ACCCGGTAGC GGGACTATCT

3201   CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC
       GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG
```

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Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
3251   TTGTTCCAAA CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA
       AACAAGGTTT GACCTTGTTG TGAGTTGGGA TAGAGCCAGA TAAGAAAACT

3301   TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA
       AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT

3351   TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GTTTACAATT
       AAATTGTTTT TAAATTGCGC TTAAAATTGT TTTATAATTG CAAATGTTAA

3401   TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT
       AGTCCACCGT GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA

3451   TTTCTAAATA CATTCAAATA TGTATCCGCT CATGTCGAGA CGTTGGGTGA
       AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACAGCTCT GCAACCCACT

3501   GGTTCCAACT TTCACCATAA TGAAATAAGA TCACTACCGG GCGTATTTTT
       CCAAGGTTGA AAGTGGTATT ACTTTATTCT AGTGATGGCC CGCATAAAAA

3551   TGAGTTATCG AGATTTTCAG GAGCTAAGGA AGCTAAAATG GAGAAAAAAA
       ACTCAATAGC TCTAAAAGTC CTCGATTCCT TCGATTTTAC CTCTTTTTTT

3601   TCACTGGATA TACCACCGTT GATATATCCC AATGGCATCG TAAAGAACAT
       AGTGACCTAT ATGGTGGCAA CTATATAGGG TTACCGTAGC ATTTCTTGTA

3651   TTTGAGGCAT TTCAGTCAGT TGCTCAATGT ACCTATAACC AGACCGTTCA
       AAACTCCGTA AAGTCAGTCA ACGAGTTACA TGGATATTGG TCTGGCAAGT

3701   GCTGGATATT ACGGCCTTTT TAAAGACCGT AAAGAAAAAT AAGCACAAGT
       CGACCTATAA TGCCGGAAAA ATTTCTGGCA TTTCTTTTTA TTCGTGTTCA

3751   TTTATCCGGC CTTTATTCAC ATTCTTGCCC GCCTGATGAA TGCTCATCCG
       AAATAGGCCG GAAATAAGTG TAAGAACGGG CGGACTACTT ACGAGTAGGC

3801   GAGTTCCGTA TGGCAATGAA AGACGGTGAG CTGGTGATAT GGGATAGTGT
       CTCAAGGCAT ACCGTTACTT TCTGCCACTC GACCACTATA CCCTATCACA

3851   TCACCCTTGT TACACCGTTT TCCATGAGCA AACTGAAACG TTTTCATCGC
       AGTGGGAACA ATGTGGCAAA AGGTACTCGT TTGACTTTGC AAAAGTAGCG

3901   TCTGGAGTGA ATACCACGAC GATTTCCGGC AGTTCTACA CATATATTCG
       AGACCTCACT TATGGTGCTG CTAAAGGCCG TCAAGATGT GTATATAAGC

3951   CAAGATGTGG CGTGTTACGG TGAAAACCTG GCCTATTTCC CTAAAGGGTT
       GTTCTACACC GCACAATGCC ACTTTTGGAC CGGATAAAGG GATTCCCAA

4001   TATTGAGAAT ATGTTTTTCG TCTCAGCCAA TCCCTGGGTG AGTTTCACCA
       ATAACTCTTA TACAAAAAGC AGAGTCGGTT AGGGACCCAC TCAAAGTGGT

4051   GTTTTGATTT AAACGTGGCC AATATGGACA ACTTCTTCGC CCCCGTTTTC
       CAAAACTAAA TTTGCACCGG TTATACCTGT TGAAGAAGCG GGGGCAAAAG
```

Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

```
4101   ACCATGGGCA AATATTATAC GCAAGGCGAC AAGGTGCTGA TGCCGCTGGC
       TGGTACCCGT TTATAATATG CGTTCCGCTG TTCCACGACT ACGGCGACCG

4151   GATTCAGGTT CATCATGCCG TCTGTGATGG CTTCCATGTC GGCAGAATGC
       CTAAGTCCAA GTAGTACGGC AGACACTACC GAAGGTACAG CCGTCTTACG

                         ScaI
                       ~~~~~~~
4201   TTAATGAATT ACAACAGTAC TGCGATGAGT GGCAGGGCGG GGCGTAATTT
       AATTACTTAA TGTTGTCATG ACGCTACTCA CCGTCCCGCC CCGCATTAAA

4251   TTTTAAGGCA GTTATTGGTG CCCTTAAACG CCTGGTGCTA CGCCTGAATA
       AAAATTCCGT CAATAACCAC GGGAATTTGC GGACCACGAT GCGGACTTAT

4301   AGTGATAATA AGCGGATGAA TGGCAGAAAT TCGAAAGCAA ATTCGACCCG
       TCACTATTAT TCGCCTACTT ACCGTCTTTA AGCTTTCGTT TAAGCTGGGC

4351   GTCGTCGGTT CAGGGCAGGG TCGTTAAATA GCCGCTTATG TCTATTGCTG
       CAGCAGCCAA GTCCCGTCCC AGCAATTTAT CGGCGAATAC AGATAACGAC

4401   GTTACCGGT TTATTGACTA CCGGAAGCAG TGTGACCGTG TGCTTCTCAA
       CAAATGGCCA AATAACTGAT GGCCTTCGTC ACACTGGCAC ACGAAGAGTT

4451   ATGCCTGAGG CCAGTTTGCT CAGGCTCTCC CCGTGGAGGT AATAATTGCT
       TACGGACTCC GGTCAAACGA GTCCGAGAGG GGCACCTCCA TTATTAACGA

4501   CGACATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG
       GCTGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC

4551   ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC
       TGGGGCATCT TTTCTAGTTT CCTAGAAGAA CTCTAGGAAA AAAAGACGCG

4601   GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG
       CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC

4651   TTTGCCGGAT CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA
       AAACGGCCTA GTTCTCGATG GTTGAGAAAA AGGCTTCCAT TGACCGAAGT

4701   GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC
       CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG

4751   CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG CTCTGCTAAT
       GTGGTGAAGT TCTTGAGACA TCGTGGCGGA TGTATGGAGC GAGACGATTA

4801   CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT
       GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA

4851   TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG
       ACCTGAGTTC TGCTATCAAT GGCCTATTCC GCGTCGCCAG CCCGACTTGC

4901   GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT
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Figure 25: pTERMsc2H10myc3sCAM: functional map and sequence (continued)

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           CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA

     4951  GAGATACCTA CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA
           CTCTATGGAT GTCGCACTCG ATACTCTTTC GCGGTGCGAA GGGCTTCCCT

     5001  GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC
           CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG

     5051  ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA GTCCTGTCGG
           TGCTCCCTCG AAGGTCCCCC TTTGCGGACC ATAGAAATAT CAGGACAGCC

     5101  GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG
           CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC

     5151  GGCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCCTG
           CCGCCTCGGA TACCTTTTTG CGGTCGTTGC GCCGGAAAAA TGCCAAGGAC

     5201  GCCTTTTGCT GGCCTTTTGC TCACATG
           CGGAAAACGA CCGGAAAACG AGTGTAC
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Table 1: Phagemids Constructed for Experiments 2 and 3

Name	FLAG	His6	gIII	Size (bp)	Insert	REN1	REN2	Resistance
pING1-A1	-	+	-	3783	His	EcoRV	SmaI	Ap
pING1-A2	-	-	-	3795	Strep-tag	EcoRV	SmaI	Ap
pING3-A1	+	+	-	3792	His	EcoRV	SmaI	Ap
pING3-A2	+	-	-	3804	Strep-tag	EcoRV	SmaI	Ap
pONG3-A	+	-	+	4278	-	EcoRV	EcoRI	Ap
pYANG3-A	+	-	+	4404	Jun	EcoRV	EcoRI	Ap
pYANG3-Ape2	+	-	+		pep2	XbaI	HindIII	Ap
pYANG3-Ape3	+	-	+		pep3	XbaI	HindIII	Ap
pYANG3-Ape10	+	-	+		pep10	XbaI	HindIII	Ap
pING1-C1	-	+	-	3853	His	EcoRV	SmaI	Cm
pING1-C2	-	-	-	3865	Strep-tag	EcoRV	SmaI	Cm
pING3-C1	+	+	-	3862	His	EcoRV	SmaI	Cm
pING3-C2	+	-	-	3874	Strep-tag	EcoRV	SmaI	Cm
pYING3-C1	+	+	-	3994	Fos	EcoRV	EcoRI	Cm
pYING3-C2	+	+	-	4315	p75	EcoRV	EcoRI	Cm
pYING3-C3	+	+	-	4240	IL-16	EcoRV	EcoRI	Cm

Table 2: Results of Experiment 2 (see Figure 7)

Table 2a: Combination of phagemids present in initial library (α)

	Combination	Clone(s)
1.	pYING1-C2 + pYANG3-ApeX	6
2.	pYING1-C1 + pYANG3-A	1
3.	pYING1-C1 + pYANG3-ApeX	1
4.	pYING1-C2 + pYANG3-A	1
5.	pYING1-C2 + ?	1

Table 2b: Combination of phagemids present after selection (β)

	Combination	Clone(s)
1.	pYING1-C2 + pYANG3-ApeX	1
2.	pYING1-C1 + pYANG3-A	9

Table 3: Results of Experiment 4 (see Figure 19)

Table 3a: Identification of phage/plasmid present in individual clones

Combination	Clone(s)
fhag1A + pUC19/IMPhag	#9
fpep3_1b + pUC18/IMP-p75	#1,#3,#5,#6,#7,#13,#15,#19
fpep3_1b + pUC19/IMPhag	#14
unusual DNA	#2,#4,#8,#10,#11,#12,#16,#17,#18

Table 3b: Test for infectivity of individual clones

Clone #	Titer (transducing units/ml)
1	2 x 10E4
2	31
3	1 x 10E5
4	1 x 10E5
5	1 x 10E5
6	2 x 10E3
7	1 x 10E4
8	1 x 10E5
9	1 x 10E6
10	1 x 10E4
11	1 x 10E3
12	1 x 10E4
13	3 x 10E3
14	< 10
15	5 x 10E4
16	1 x 10E4
17	5 x 10E2
18	1 x 10E4
19	1 x 10E5

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/10   C12N15/12   C12N15/24   C12N15/62   C12N15/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	EP 0 614 989 A (MORPHOSYS GES FUER PROTEINOPTI) 14 September 1994 cited in the application see the whole document ---	1-34
A	DE 41 22 598 C (DKFZ) 30 July 1992 see the whole document --- -/--	1-34

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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